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L'influence de la méiofaune sur le fonctionnement du biofilm lotique en relation avec la qualité de l'eau

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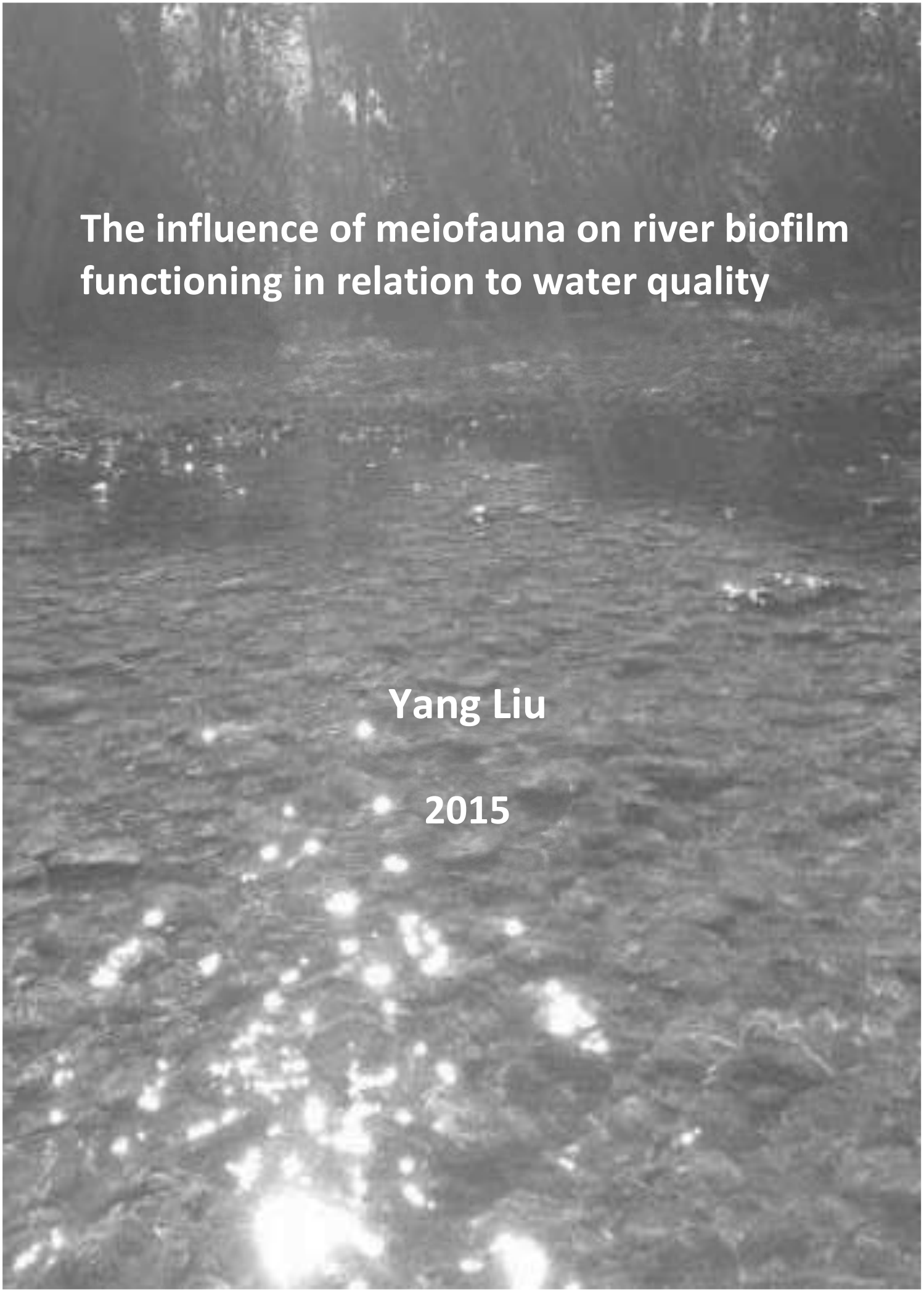

The influence of meiofauna on river biofilm functioning in relation to water quality

Yang Liu

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Foreword

This thesis includes 6 chapters, among which Chapter 1 (Introduction) and Chapter 6 (General discussion and conclusion) are preceded by French translations. The literature references are listed at the end of this thesis.

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2. **Liu Y.**, Tackx M., Dauta A., Julien F., Buffan-Dubau E. Interaction between biofilm-dwelling rotifers and bacteria increases N-NO_3^- biofilm uptake rate. Freshwater Biology. *Submitted, in revision*
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Besides, I developed a tool for bibliometric analysis by R. Welcome to use it!
https://yangliufr.shinyapps.io/a-bibliometric-tool

I cooperated with my colleagues to practice this tool, and achieved two publications:

1. Wang C., **Liu Y.,** Li X., Lai Z., Tackx M., Lek S. (2015) A bibliometric analysis of scientific trends in phytoplankton research. Annales de Limnologie - International Journal of Limnology 51 (3), 249-259
2. Guo C., Park Y., **Liu Y.**, Lek S. (2015) Toward a New Generation of Ecological Modelling Techniques: Review and Bibliometrics, in Advanced Modelling Techniques Studying Global Changes in Environmental Sciences, 1st Edition, Elsevier, Chapter 2, 11-44

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List of abbreviations

AFDM	: Ash-free dry mass
AS	: Abiotic sediment
C	: Carbon
Chl. *a*	: Chlorophyll *a*
Chl. *b*	: Chlorophyll *b*
DIN	: Dissovled inorganic nitrogen
DM	: Dry mass
DNRA	: Dissimilatory nitrate reduction to ammonium
DOC	: Dissolved organic carbon
DOM	: Dissolved organic matter
EPS	: Exopolymeric substances
HPLC	: High-performance liquid chromatography
ind.	: Individuals
N	: Nitrogen
NAT	: Natural river water
NUT	: Nutrient enriched natural river water
NAT-BIOF	: NAT with biofilm
NUT-BIOF	: NUT with biofilm
NUT-BIOF+	: NUT with biofilm and meiofauna
SB	: Sediment and biofilm
SBM	: Sediment, biofilm and meiofauna
SBMM	: Sediment, biofilm, meiofauna and macrofauna
SBT	: SB with toxic (diuron)
SBMT	: SBM with toxic (diuron)
SBMMT	: SBMM with toxic (diuron)
SRP	: Soluble reactive phosphorus
ST	: Sediment with toxic (diuron)

Chapter 1: Introduction

1.1 Version française

1.1.1 Les biofilms lotiques

Les biofilms lotiques sont des communautés complexes d'organismes (bactéries, micro-algues, hyphomycètes, protozoaires, petits invertébrés) associés dans une matrice d'exopolymères (EPS) produits par sa fraction microbienne (Neu et al., 2003). Ils se développent sur tout substrat immergé ou exposé à une solution aqueuse (e.g. Jones & Lock, 1993; Neu et al., 2003; Costerton, 2010; Majdi et al., 2012a), sur substrats durs, il est nommé biofilm épilithique ou épilithon (Hill et al., 1996). Les biofilms exposés à la lumière comprennent une communauté microbienne constituée à la fois d'hétérotrophes et de phototrophes associés dans leur matrice d'EPS (Haack & Mcfeters, 1982; Lock et al., 1984).

La recherche sur les biofilms lotiques auraient été initiée dans les années 1970 (Weitzel, 1979) montrant ses progrès les plus significatifs dans les 30 dernières années. Une partie de ces etudes a ont été focalisée sur la formation et la micro-architecture des biofilms. Les réacteurs annulaires rotatifs (RAR) sont par exemple utilisés dans le but de cultiver des biofilms complexes lotiques (Neu & Lawrence, 1997; Lawrence et al., 1998) tandis que la microscopie confocale (2-PLSM) est très appliquée à l'étude d'images tridimensionnelles d'échantillons de biofilms vivants et totalement hydratés (Neu et al., 2002; 2003). Par ailleurs, l'étude des interactions complexes intervenant entre les différents groupes associés aux biofilms (bactéries - algues - invertébrés) et leur organisation (e.g. réseaux trophiques, minéralisation de la matière organique et production primaire), constitue aussi un axe majeur de ces recherches (Schmid Araya & Schmid, 2000; Neu et al., 2003; Liess & Hillebrand, 2004; Majdi et al., 2012b).

Dans les cours d'eau, les substrats favorables à la croissance des biofilms sont trouvés (1) à l'interface eau-sédiment (zone benthique) plus ou moins exposée à la lumière, et (2) sur les particules sédimentaires non exposées à la lumière (Barlocher & Murdoch, 1989; Claret, 1998a; Koutny & Rulik, 2007). Les biofilms benthiques (phototrophes ou hétérotrophes) et les biofilms hyporhéiques (hétérotrophes) dominent la communauté microbienne dans les écosystèmes caractérisés par un grand rapport entre la surface des sédiments et le volume d'eau comme les rivières par exemple (Battin et al., 2001; 2008; Marmonier et al., 2012).

1.1.1.1 Les biofilms phototrophes

Les biofilms phototrophes (ou periphyton) dépendent de l'énergie lumineuse puisqu'ils possèdent un compartiment photosynthétique important. Ils comprennent notamment des micro-organismes photosynthétiques oxygéniques tels que des diatomées, des micro-algues vertes et des cyanobactéries qui puisent une partie du CO_2 et des nutriments dans l'eau surnageante et produisent le carbone organique nécessaire à la vie de la fraction hétérotrophe de la communauté microbienne, principalement des bactéries (Roeselers et al., 2008; Buhmann et al., 2012) mais aussi des hyphomycètes et des ciliés (Barlocher, 1987; Norf et al., 2009), et produisent en même temps l'oxygène. Les hétérotrophes obtiennent leur carbone et azote organiques à partir de particules collectées, à partir des excrétas libérés par les organismes photosynthétiques ou des produits de lyses cellulaires, et, contribuent positivement à la régénération des nutriments (Bateson & Ward, 1988). La matrice polymérique des biofilms peut servir de refuge à ces communautés microbiennes et aux petits invertébrés, contre les forces de cisaillement hydrauliques (Lock, 1993; Gaudes et al., 2006; Majdi et al., 2012a).

1.1.1.2 Les biofilms hétérotrophes

Les environnements obscurs des cours d'eau sont présents (1) lorsque la colonne d'eau ne favorise pas la pénétration de la lumière (e.g. dans les rivières turbides, les rivières à forte pollution organique, les rivières eutrophisées présentant des proliférations algales) ; (2) dans les zones hyporhéiques, zones de connexion entre les eaux de surface et les eaux souterraines (Barlocher & Murdoch, 1989; Jones & Lock, 1993; Mohamed et al., 1998; Findlay & Sinsabaugh, 2003). Les sédiments des zones benthiques et hyporhéiques offrent une vaste aire favorable à la croissance des biofilms hétérotrophes (Battin et al., 2001).

1.1.1.3 Rôle des biofilms dans les écosystèmes lotiques

Les biofilms phototrohes et hétérotrophes peuvent former une matrice cohésive qui englobe et piège les particules assurant ainsi un rôle important contre la remise en suspension et dans la stabilisation des sédiments (Gerbersdorf & Wieprecht, 2015). Les micro-organismes associés aux biofilms participent au recyclage de la matière détritique (par minéralisation et consommation), peuvent consommer l'azote et recycler l'azote organique, et puiser l'énergie et le carbone par photosynthèse et chimiosynthèse à la fois à partir de l'eau environnante (sources allochtones) et à partir du biofilm lui-même (sources autochtones) (Kuserk et al., 1984; Findlay et al., 1986; Romaní, 2009).

En milieu lotique, la matière organique détritique (MOD) est la forme dominante de la matière organique, comparée à la matière organique particulaire (MOP). Une large part de MOD comprend des substances humiques et polymériques (Volk et al., 1997). La communauté microbienne utilise à la fois des sources de MOD labile et une partie de MOD réfractaire (Norrman et al., 1995). La biodisponibilité du carbone organique dissous (COD) est importante pour les taux de consommation et les composés organiques des biofilms hétérotrophes en particulier (Docherty et al., 2006). Le COD et les biofilms sont des sources importantes d'énergie pour les communautés lotiques (Simon et al., 2003) donc, la fraction hétérotrophe des biofilms joue un rôle important dans le recyclage de la MOD des cours d'eau (Sabater et al. 2002). En outre, les biofilms sont parmi les principaux producteurs primaires (Vadeboncoeur & Steinman, 2002), comme par exemple dans la zone moyenne de la Garonne (le troisième plus grand fleuve de France recouvrant 57000 km^2), où la vitesse du courant relativement élevée n'est pas favorable au développement du plancton (Ameziane et al., 2003; Leflaive et al., 2008).

L'eutrophisation des écosystèmes d'eau douce représente une préoccupation majeure de « santé écologique » des eaux de surface, en particulier en régions agricoles (Krause et al., 2009). Les activités anthropiques influencent les concentrations en nitrates relevées dans les cours d'eau européens. Lassaletta et al. (2009) ont suivi l'évolution des concentrations en nitrates pendant 25 ans (1981-2005) dans le bassin de la rivière Ebre (Espagne) couvrant 85 566 km^2. Ils ont montré que les concentrations moyennes en nitrates varient de 1,3 à 40,3 mg L^{-1}, toutes supérieures à 0,44 mg L^{-1}, la concentration de référence proposée par Meybeck (1982) pour les principaux cours d'eau non pollués. Dans un aquifère peu profond de la Garonne situé à 50 km en aval de Toulouse, la concentration moyenne en $N\text{-}NO_3$ était de 10,2 ± 1,9 mg L^{-1} entre 2000 et 2004, ce qui indique que cet aquifère est sévèrement pollué (Iribar et al., 2007). Les conséquences néfastes produites par des concentrations excessives en nutriments des eaux de surface et des eaux souterraines, sur la structure et le fonctionnement des écosystèmes sont bien établies (Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999). De récents travaux suggèrent que la biorémédiation (i.e. réduction des pollutions par des processus biologiques dont les métabolismes microbiens) par les biofilms benthiques constitue une voie prometteuse pour faire face à l'eutrophisation (Singh et al., 2006; Sun et al., 2009; Cao et al., 2012). En effet, les biofilms et particulièrement les biofilms phototrophes peuvent agir comme des « puits » à nutriments pour la colonne d'eau, renforçant ainsi les échanges verticaux et horizontaux des ressources et contribuer aux processus « d'auto-épuration » des écosystèmes lotiques (Pusch et al., 1998; Sabater et al.,

2002; Battin et al., 2003; Teissier et al., 2007). Cette capacité d'auto-épuration des biofilms est la base essentielle de ce travail de thèse.

Les processus favorisant la réduction des concentrations en nitrate au sein des écosystèmes sont complexes et il est difficile d'en établir une liste exhaustive. La figure 1-1 présente une schématisation générale de ces processus proposée par Burgin & Hamilton (2007). L'assimilation des nitrates par la biomasse photosynthétique (produisant l'azote organique) et la dénitrification par les bactéries (produisant du N_2) sont deux voies bien identifiées. La réduction dissimilatrice des nitrates en ammonium (DNRA) est une des voies alternatives à la dénitrification, elle peut impliquer par exemple la fermentation et l'oxydation du sulfure. Par ailleurs, la dénitrification liée à l'oxydation du fer (produisant du NO_2) et l'oxydation anaérobique de l'ammonium (« Anammox », produisant du N_2) sont deux autres voies importantes mais moins bien connues que celles décrites précédemment. Les micro-organismes participent activement à ces processus. Parmi ces voies de réduction des concentrations en nitrates, la dénitrification est le processus majeur réduisant les charges en nitrates des écosystèmes lotiques qui sont déversées dans le milieu marin côtier (Galloway et al., 2003). Les bactéries dénitrifiantes sont présentes et actives à la fois dans les biofilms phototrophes et dans les biofilms hétérotrophes de zones hyporhéiques (Pinay et al., 2009; Lyautey et al., 2013). Iribar et al. (2007) ont étudié l'implication des communautés bactériennes libres et fixées de la Garonne dans les processus de dénitrification. Les auteurs indiquent que la capacité dénitrifiante bactérienne était présente au sein des communautés microbiennes fixées alors qu'elle ne l'était pas chez celles vivant en suspension dans les eaux souterraines.

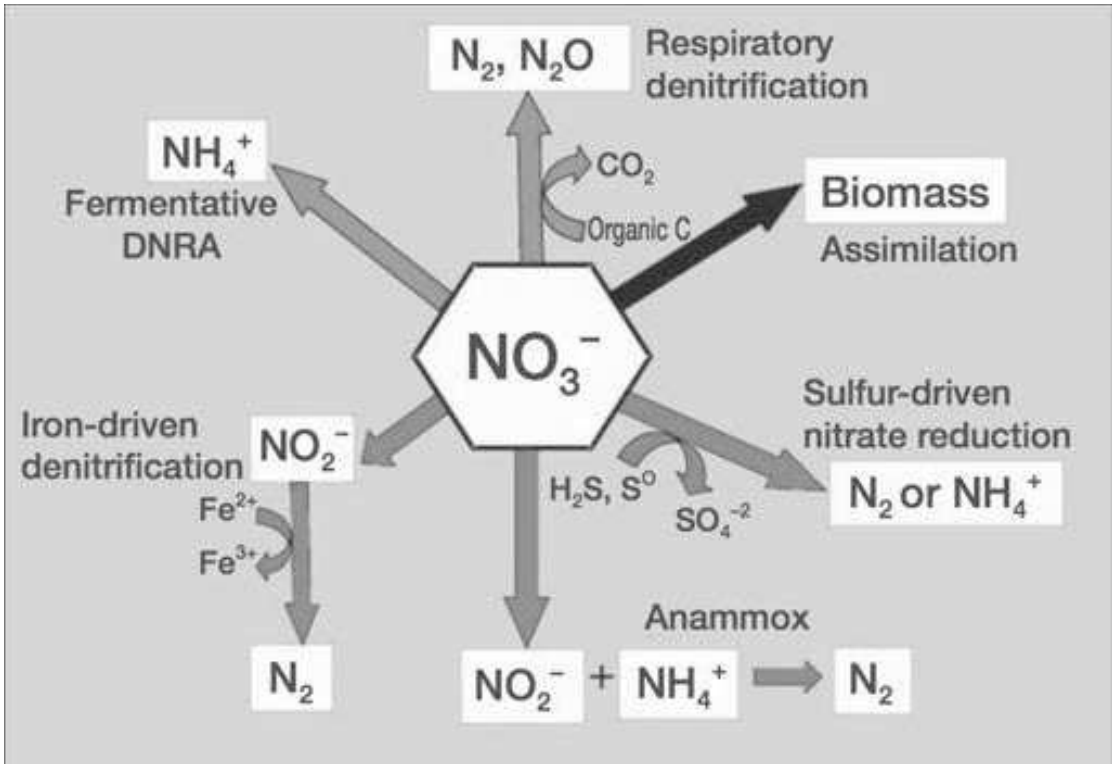

Figure 1.1 Les différentes voies de réduction des concentrations en nitrates dans les écosystèmes aquatiques d'après Burgin & Hamilton 2007. Les flèches bleues indiquent les processus autotrophes et les flèches roses indiquent les processus hétérotrophes.

Les polluants organiques (e.g. herbicides) sont cummulatifs en milieu hyporhéique étant donné la surface importante d'adhésion fournie par les sédiments de cette zone (Hedges & Keil, 1995; Pereira et al., 1996; Devault et al., 2009). Le diuron (N-[3,4-dichlorophenyl]-N,N-dimethylurea; CAS No. 330-54-1) est un herbicide à large spectre résiduel. Les concentrations en diuron des cours d'eau européens varient de 2.1 à 36 µg L^{-1} (López-Doval et al., 2009). Cet herbicide peut inhiber la photosynthèse chez les algues et les cyanobactéries en limitant la production d'ATP nécessaire aux processus métaboliques microbiens (Corbett, 1984; Hayes, 1991; Pesce et al., 2006; Ricart et al., 2009). Etant donné que le biofilm hyporhéique n'est pas photosynthétique, il peut ne pas être affecté par le diuron (Proia et al., 2011). Cependant, les métabolismes micobiens des biofilms étant très diverses, ils pourraient etre capables d'interagir avec les herbicides tels que le diuron (Pesce et al., 2009; Vercraene-Eairmal et al., 2010; Pesce et al., 2013).

1.1.2 La méiofaune

1.1.2.1 Définition

Le terme méiofaune a été introduit par Mare (1942). L'intervalle de tailles utilisé pour définir la méiofaune peut varier en fonction des auteurs: 44-500 µm selon Higgin and Thiel

(1988), 42-500 et 63 µm-1000 µm d'après Giere (2009). La méiofaune regroupe les invertébrés benthiques mobiles et haptosessiles dont la taille est supérieure à celle de la microfaune et inférieure à celle de la macrofaune (Giere, 2009). Il peut aussi être considéré que les grands protozoaires (e.g. ciliées, amibes) dont la taille entre dans les intervalles donnés plus haut, appartiennent à la méiofaune (Giere, 2009). On distingue la méiofaune « permanente » de la méiofaune « temporaire » regroupant les individus dont la taille correspond à celle de la méiofaune seulement pendant une partie de leur développement (par exemple les larves d'insectes). La Fig. 1-2 montre les principaux groupes méiobenthiques observés dans les biofilms de la Garonne; les nématodes, rotifères et larves de chironomidae. La méiofaune est abondante dans tous les écosystèmes; les sols, les écosystèmes d'eau douce et marins (Murphy, 1958; Gerlach, 1971; Rundle et al., 2002). La présence de la méiofaune dans les biofilms paraît aussi ubiquiste (Gaudes et al., 2006; Kathol et al., 2011; Majdi et al., 2012a; Carpentier et al., 2014).

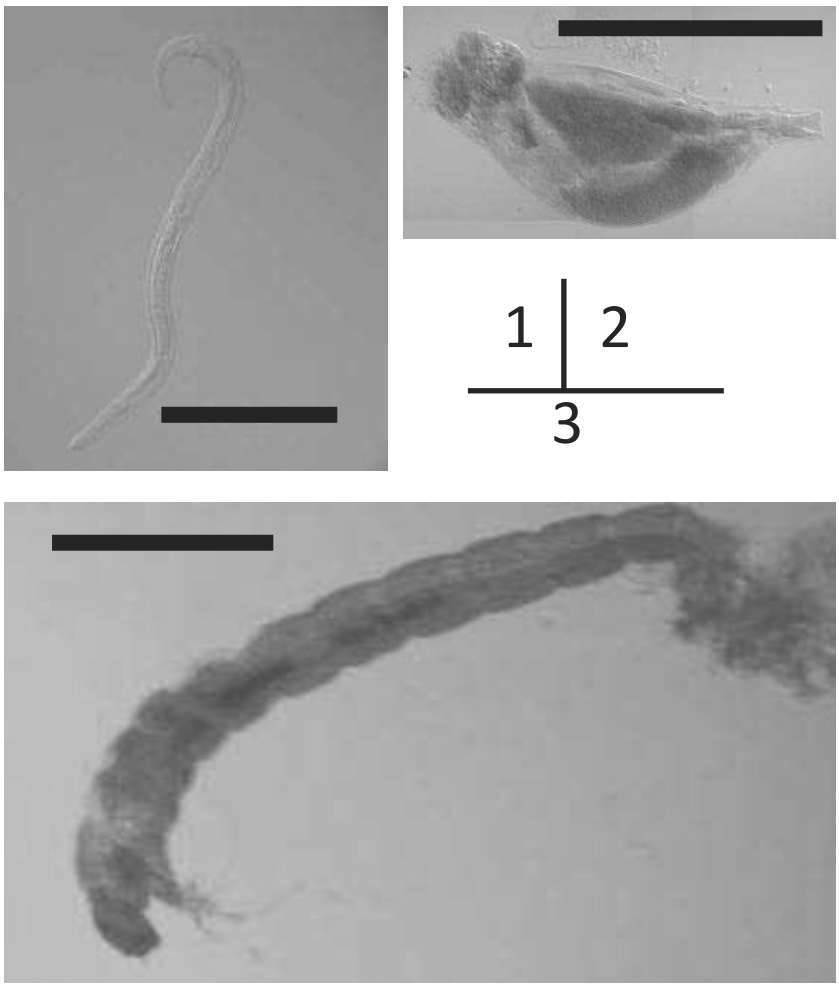

Figure 1-2 Les trois principaux groupes méiobenthiques observés dans les biofilms de la Garonne: 1, nématode (*Chromadorina* sp.) ; 2, rotifère (*Proales* sp.) ; 3, larve de chironomidae . Les photos sont de B. Mialet, N. Majdi, et F. Azémar (Ecolab, UPS Toulouse). Une barre-échelle = 200 µm.

1.1.2.2 La méiofaune associée aux biofilms

Il existe de nombreux travaux portant sur les invertébrés et les biofilms. Par exemple Hillebrand (2009) examine 835 études consacrées au contrôle de la biomasse du biofilm périphytique par la consommation de la macrofaune. Toutefois, il est surprenant de constater que la méiofaune associée aux biofilms a été sous-étudiée. L'abondance des nématodes peut par exemple atteindre 10^5-10^6 ind. m^{-2} dans la matrice des biofilms cyanobactériens (Farmer, 1992; Gaudes et al., 2006) et 3×10^6 ind. m^{-2} dans les biofilms (dominés par les diatomées) de la Garonne (Majdi et al., 2012a). La colonisation des biofilms par la méiofaune est taxon spécifique, par exemple, après les périodes de crue, les rotifères sont des colonisateurs plus rapides que les nématodes dans le biofilm épilithique de la Garonne, et, leur densité dépend de la biomasse du biofilm (Majdi et al., 2012a). De plus, les rotifères bdelloides ont une glande qui sécrète une substance adhésive qu'ils utilisent pour se fixer temporairement au substrat (Ricci & Balsamo, 2000), ce qui leur confère une capacité à résister au courant relativement élevée (Majdi et al., 2012a). La diversité de la méiofaune associée au biofilm est habitat spécifique. Dans la Garonne par exemple, les nématodes et les rotifères sont les taxons dominants en terme de densité, suivis par les chironomidae, les oligochètes, les copépodes harpacticoïdes et les tardigrades (Majdi et al., 2012a). Dans les biofilms de sédiments vaseux côtiers (France), les taxons pricipaux observés sont les nématodes, les copépodes, les foraminifères, les ostracodes et les plathelminthes (Carpentier et al., 2014) alors que dans un cours d'eau de second ordre de la basse Autriche, les copépodes harpacticoïdes, les ostracodes et les nématodes sont les groupes dominants (Schmid Araya, 2000). En général, l'activité trophique de la méiofaune n'induit pas de réduction significative de la biomasse des biofilms (Majdi et al., 2012b; 2012c). Toutefois, Mialet et al. (2013) en étudiant les contenus pigmentaires intestinaux de rotifères bdelloides collectés dans la Garonne, ont montré que les rotifères peuvent ingérer une fraction substantielle (jusqu'à 28%) de la biomasse cyanobactérienne du biofilm de la Garonne, en mettant en évidence leur comportement trophique hautement sélectif.

1.1.2.3 Rôle de la méiofaune dans le cycle de l'azote

Plusieurs auteurs ont étudié la réponse de la méiofaune exposée à des concentrations excessives en nutriments. Les résultats divergent. Hillebrand et al. (2002) n'ont observé aucun effet significatif des enrichissements en nutriments sur la biomasse de la méiofaune de sédiments lacustres ou côtiers. Par ailleurs, la réponse à long-terme (sur 5 ans) de la méiofaune (en terme de densité) de sédiments de marais maritimes, a été décrite comme

contradictoire et variable (Mitwally & Fleeger, 2013). En revanche, la méiofaune lotique (biomasse ou densité) a répondu de façon positive à des apports en nutriments (1) dans un cours d'eau (Gaudes et al., 2012) et (2) en microcosmes (Ristau et al., 2013). L'effet des nutriments sur la méiofaune associée aux biofilm n'est donc pas clairement élucidé.

En plus de la question des effets des nutriments sur la méiofaune, se pose aussi la question de l'effet potentiel de l'activité de la méiofaune sur le cycle des nutriments. La majorité des études portant sur l'effet des invertébrés sur le cycle des nutriments, comme l'azote par exemple, a considéré la macrofaune (de taille > 1mm), car d'une part elle est plus facile à manipuler en laboratoire, et d'autre part, elle affecte les processus microbiens par son activité de bioturbation. Le rôle important du macrobenthos dans la régulation de la minéralisation du carbone, de la régénération des nutriments et des processus couplés nitrification/dénitrification, est bien établi (Aller, 1994; Lillebø et al., 1999; Gerino et al., 2003; Gilbert et al., 2003; Welsh, 2003). La macrofaune peut agir sur les processus de dénitrification soit avec (1) un effet positif par ses activités fouisseuses modifiant les sédiments en créant des galeries notamment, par ventilation et bio-irrigation (Karlson et al., 2007; Stief, 2013) ou (2) un effet négatif par la voie de la réduction dissimilatrice des nitrates en ammonium (Bonaglia et al., 2013). Cependant, très peu d'études concernent les effets de la méiofaune sur le cycle des nutriments. Par exemple sur le cycle de l'azote, à notre connaissance, seules quatre études en dehors du présent travail de thèse, traitent cette question (Table 1-1).

Tableau 1-1 Synthèse des travaux antérieurs étudiant les effets potentiels de la méiofaune sur les nitrates.

Méiofaune (taxon dominant)	Habitat	Effet sur la concentration en nitrate	Processus observé	Références
Nématodes	Marin, sédiments	−	Denitrification	Hentschel et al. 1999
Copépodes	Marin, sédiments	+	Nitration	Parent et al. 1999
Nematodes	Marin, sédiments	−	Denitrification	Bonaglia et al. 2014
Copépodes	Marin, sédiments	−	Réduction dissimiatrice	Stock et al. 2014

Parent et al. (1999) ont montré que dans des sédiments marins, la biomasse de la méiofaune dominée par des copépodes (< 0,16 g m^{-2}) augmente le taux de nitrification d'un facteur deux à cinq. Les auteurs expliquent ce résultat en suggérant que lorsque la méiofaune

est faiblement représentée (en terme de biomasse), les bactéries qu'elle consomme sont essentiellement hétérotrophes tandis que lorsqu' elle est présente avec des valeurs de biomasse relativement plus élevées, elle consomme aussi bien des hétérotrophes que des bactéries nitrifiantes. D'autre part, Hentschel et al. (1999) ont montré que les nématodes appartenant à la famille Stilbonematinae peuvent être associés à des bactéries ecotsymbiontes qui sont capables de réaliser la dénitrification.

Récemment, Bonaglia et al. (2014) rapportent que l'activité de « méio-bioturbation » peut avoir un effet stimulant sur la nitrification et la dénitrification dans des sédiments marins (Fig. 1-3). Les auteurs suggèrent que l'augmentation de la production de diazote n'est pas due à une respiration directe des nitrates par la méiofaune, comme cela a pu être montré au préalable pour des espèces de foraminifères (Risgaard-Petersen et al., 2006; Hogslund et al., 2008), mais due à une bioturbation intense résultant de l'activité de la méiofaune.

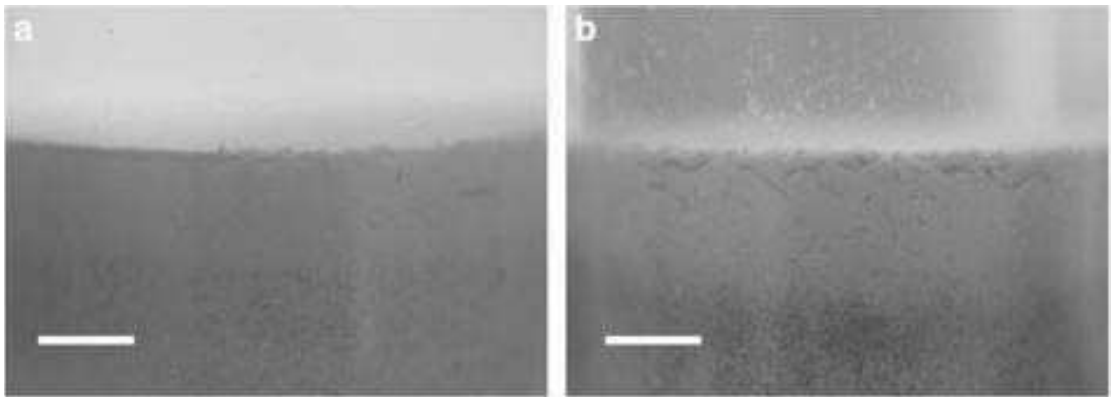

Figure 1-3 Photographies (Bonaglia et al. 2014) obtenues à l'aide d'une caméra digitale, montrant différentes intensités de « méio-bioturbation» dans la couche superficielle des sédiments de microcosmes (carottes de sédiments marins) issues (a) d'un traitement à faible densité de méiofaune et (b) d'un traitement à densité de méiofaune élevée. Une barre-échelle = 500 μm.

Stock et al. (2014) ont récemment étudié les interactions entre les copépodes benthiques, les bactéries et les diatomées dans des sédiments intertidaux. Il en résulte que les copépodes marins peuvent augmenter la DNRA plutôt que la dénitrification. L'hypothèse émise est qu'il s'agirait d'un effet indirect lié à l'excrétion des copépodes qui fournirait du carbone organique supplémentaire à la communauté bactérienne. Stock et al. (2014) n'ont cependant pas observé une réelle réduction des concentrations en nitrates suggérant que la réduction de la dénitrification par les copépodes a été compensée par un autre processus aboutissant aussi à la diminution des quantités de nitrates (Fig. 1-4).

Dans l'ensemble il apparaît que les interactions entre la méiofaune et les microorganismes pourraient avoir une influence importante sur le cycle de l'azote, dans les écosystèmes aquatiques.

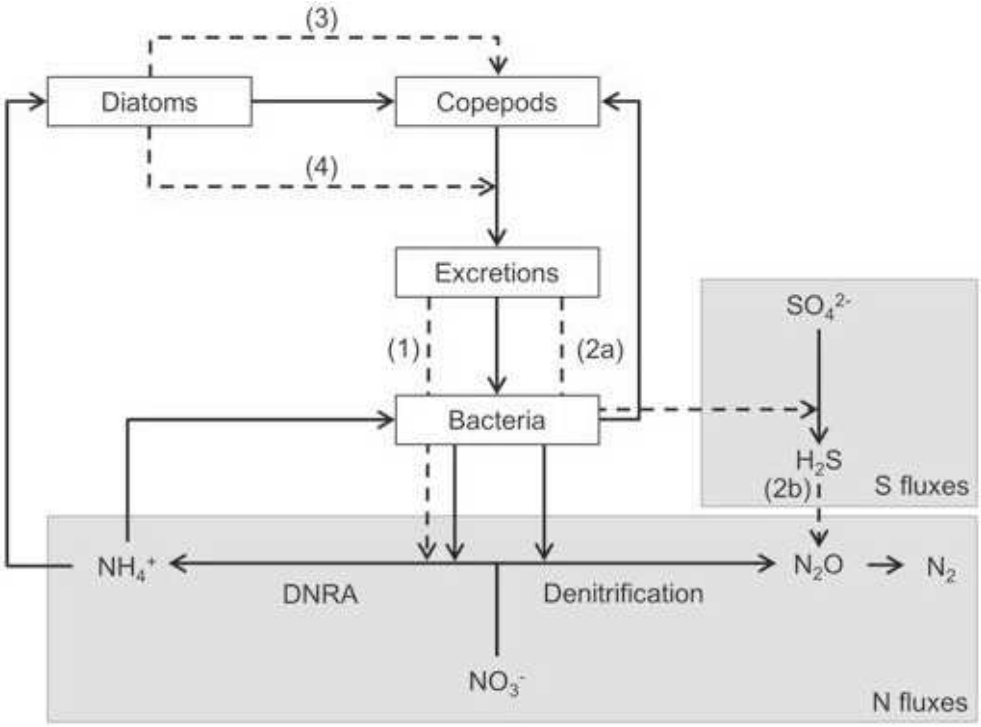

Figure 1-4. Synthèse des interactions supposées (flèches en pointillés) entre les copépodes méiobenthiques, les bactéries et les diatomées d'après Stock et al. (2014) ayant une influence sur le cycle de l'azote et le cycle du soufre de sédiments marins. Les zones grisées distinguent les flux d'azote et les flux de soufre. Les copépodes méiobenthiques consomment à la fois des diatomées et des bactéries, et, produisent des excrétions organiques utilisées par les bactéries. La communauté bactérienne intervient dans la réduction du SO_4^{2-} en H_2S et du NO_3^- en NH_4^+ (DNRA) et N_2O + N_2 (denitrification). Le NH_4^+ produit est assimilé par les diatomées et les bactéries. Les copépodes affectent le taux de production de N_2O par excrétion de produits qui apportent une source supplémentaire de carbone stimulant principalement l'activité des bactéries impliquées dans la DNRA (1) et les bactéries sulfato-réductrices (2a). L'augmentation de la concentration en H_2S inhibe la dénitrification (2b). Les diatomées n'ont pas d'effets directs sur le taux de production de N_2O mais ont un effet indirect en stimulant la croissance des copépodes (3) et influençent la quantité et la composition des produits d'extraction de ces derniers (4).

1.1.2.4 Objectifs et organisation de la thèse

Dans ce contexte, l'hypothèse testée par ce travail de thèse est que l'activité (trophique et bioturbation) de la méiofaune associée aux biofilms peut être un facteur qui influence positivement le fonctionnement du biofilm concernant en particulier le cycle de l'azote, en relation avec la qualité de l'eau. Cette étude concerne non seulement les biofilms phototrophes se développant à la surface des sédiments mais aussi les biofilms hétérotrophes présents dans le milieu hyporhéique où la méiofaune peut être abondante (Schmid Araya, 2000; Schmid Araya & Schmid, 2000). De plus, en accord avec l'hypothèse générale qui considère que la biodiversité contribue positivement au fonctionnement des écosystèmes

(Loreau et al., 2001), la macrofaune a aussi été prise en compte pour augmenter le gradient de biodiversité considéré dans les systèmes hyporhéiques. Il s'agit dans ce cas, de tester l'hypothèse d'une augmentation potentielle de la consommation de nutriments par les biofilms lorsque la diversité de la communauté présente augmente.

Dans le but d'examiner le rôle de la méiofaune associée aux biofilms dans le cycle de l'azote dans les écosystèmes benthiques et hyporhéiques, quatre études expérimentales ont été conduites en laboratoire.

La première étude (Chapitre 2) a été la première étape de ce travail visant à tester si la méiofaune des biofilms peut répondre à court-terme, à un enrichissement du milieu en nutriments, en relation avec la dynamique de consommation des nitrates par les biofilms. Cette étude expérimentale a été réalisée à partir de biofilms phototrophes complexes cultivés en milieu naturel.

Etant donné qu'une réponse significative de la méiofaune a été observée dans la première étude et que les résultats suggéraient un lien possible entre les interactions méiofaune-bactéries et la capacité de consommation de l'azote par le biofilm à court-terme, la seconde étude (Chapitre 3) a porté sur l'effet de la densité de la méiofaune sur la consommation de l'azote par les biofilms phototrophes au sein de microcosmes.

La méiofaune présente dans les biofilms hyporhéiques (hétérotrophes) a été étudiée par les travaux décrits dans les chapitres 4 et 5. Dans le Chapitre 4, le rôle de la méiofaune sur la consommation de l'azote par les biofilms hétérotrophes a été testé au sein de colonnes sédimentaires, en microcosmes. De plus, étant donné que les micro-organismes qui participent aux cycles des nutriments et à la transformation de la matière organique sont soumis au contrôle *top-down* exercé par les niveaux trophiques supérieurs, cette étude dans son ensemble examine le rôle de la diversité d'un assemblage multi-communautaire (biofilm-méiofaune-macrofaune) sur la consommation des nitrates et du carbone organique dissous.

La quatrième étude (Chapitre 5) a été réalisée dans les mêmes conditions que l'expérience décrite dans le chapitre 4, cependant, des traitements contenant un herbicide (le diuron) ont été ajoutés au design expérimental. Il s'agissait de tester si les invertébrés ont la capacité de modifier la perturbation potentielle causée par le diuron, sur la consommation de l'azote par le biofilm. Tandis que j'ai entièrement mené les études décrites dans les chapitres 2 et 3, ma contribution aux études des Chapitres 4 et 5 a été focalisée sur l'analyse des résultats obtenus et la rédaction de manuscrits.

1.2 Version anglaise

1.2.1 Biofilm in rivers

Biofilms occurring in rivers are complex aggregations of microorganisms e.g. bacteria, algae, fungi and heterotrophic protozoans enclosed in an exopolymeric matrix (EPS, Extracellular Polymeric Substances) and growing on substrates submerged in or exposed to some aqueous solution (e.g. Jones & Lock, 1993; Neu et al., 2003; Costerton, 2010; Majdi et al., 2012a). Epilithic biofilm (epilithon) develops on hard substrates (Hill et al., 1996). When (partially) exposed to light, the attached microbial communities generally includes phototrophic and heterotrophic organisms in a polymeric matrix (Haack & Mcfeters, 1982; Lock et al., 1984).

The investigation of biofilms in lotic systems started in the seventies (Weitzel, 1979) and made much progress within the last 30 years. Some studies focused on the development and architecture of biofilms. For instance, rotating annular reactors (RAR) have been used to grow complex lotic biofilms in order to quantify biofilm parameters (Neu & Lawrence, 1997; Lawrence et al., 1998), and confocal laser scanning microscopy (CLSM) and 2-photon laser scanning microscopy (2-PLSM) is very well suited for the 3-dimensianl imaging of living, fully hydrated biofilm samples (Neu et al., 2002; 2003). Besides, complex interactions among groups of organisms associated with biofilms (e.g. bacteria - algae - invertebrates) and pathways (e.g. food web, detritus processing, and primary production) were also considered (Schmid Araya & Schmid, 2000; Neu et al., 2003; Liess & Hillebrand, 2004; Majdi et al., 2012b).

In rivers, the substrates for biofilm growth can be found (1) at the water-sediment interface (benthic zone) exposed to daylight, and (2) on sedimentary particles in the absence of light (Barlocher & Murdoch, 1989; Claret, 1998a; Koutny & Rulik, 2007). Phototrophic benthic biofilms and heterotrophic hyporheic biofilms typically dominate microbial life in ecosystems with large sediment-surface-area to water-volume ratios e.g. rivers (Battin et al., 2001; 2008; Marmonier et al., 2012).

1.2.1.1 Phototrophic biofilms

Phototrophic biofilms (or periphyton) are driven by light energy with a photosynthesizing component clearly present. They comprise oxygenic photoautotrophic microorganisms such as benthic diatoms, green algae and cyanobacteria, which take up CO_2 and nutrients from the water to produce the organic carbon that fuels the life of a heterotrophic contingent of microorganisms, mostly bacteria (Roeselers et al., 2008;

Buhmann et al., 2012) as well as hyphomycetes and ciliates (Barlocher, 1987; Norf et al., 2009), meanwhile producing oxygen. Heterotrophs derive their organic C and N requirements from either filtered particles or excreted photosynthates and cell lysates, and positively contribute to nutrient regeneration (Bateson & Ward, 1988). The polysaccharide matrix of biofilms can provide a refuge for these microbial communities and invertebrates from hydraulic shear forces (Lock, 1993; Gaudes et al., 2006; Majdi et al., 2012a).

1.2.1.2 Heterotrophic biofilms

Dark environments in rivers can be realized by (1) river water with low light penetration (e.g. turbid or muddy rivers; polluted rivers; and over nourished river with algae blooms); (2) hyporheic zones connecting surface water and ground water (Barlocher & Murdoch, 1989; Jones & Lock, 1993; Mohamed et al., 1998; Findlay & Sinsabaugh, 2003). The sediments on/beneath the riverbed offer a large surface area for colonization by heterotrophic biofilms (Battin et al., 2001).

1.2.1.3 The role of biofilms in lotic ecosystems

Both phototrophic and heterotrophic biofilms can form a cohesive matrix closely surrounding and embedding particles, which can have an important role in stabilizing sediments against re-suspension (Gerbersdorf & Wieprecht, 2015). the attached microorganisms within biofilms can recycle organic detritus, decompose and take up organic matter, fix nitrogen or recycle organic nitrogen, and fix energy and carbon by photosynthesis and chemosynthesis, both from the surrounding water (allochthonous sources) and from within the main biofilm (autochthonous sources) (Kuserk et al., 1984; Findlay et al., 1986; Romaní, 2009).

In rivers, dissolved organic matter (DOM) is the dominant fraction of organic matter, compared to particulate organic matter (POM), and a large part of DOM is made up of humic substances and polymeric molecules (Volk et al., 1997). Microbial communities will utilize labile DOM sources as well as parts of refractory DOM (Norrman et al., 1995). The bioavailability of dissolved organic carbon (DOC) for the biofilm heterotrophs is important for their uptake rates of organic compounds (Docherty et al., 2006). DOC and biofilms are important energy sources for stream communities (Simon et al., 2003). Therefore, heterotrophic fractions of biofilms have an important role in recycling DOM in rivers (Sabater et al., 2002). Besides, phototrophic biofilms are one of the main primary producers (Vadeboncoeur & Steinman, 2002), as for example in the medium part of Garonne River

(SW France) where the high stream velocity makes the system unsuitable for plankton growth (Ameziane et al., 2003; Leflaive et al., 2008).

Eutrophication of freshwater environments represents a major threat to the ecological health of surface waters in catchments, especially near agricultural regions (Krause et al., 2009). Anthropogenic activities influence nitrate levels recorded in European stream waters in the past and at present. Lassaletta et al. (2009) studied the evolution of nitrate concentrations over 25 years (1981-2005) in the Ebro River Basin (Spain), a large Mediterranean catchment covering 85,566 km^2. They have shown that the average NO_3 concentrations ranged from 1.3 to 40.3 mg L^{-1}, all of which exceeded 0.44 mg L^{-1} of NO_3, the background concentration proposed by Meybeck (1982) for the major unpolluted rivers. In a shallow aquifer of a riparian zone of the Garonne River, the third largest in river France, (57,000 km^2), 50 km downstream of Toulouse city, the mean N-NO_3 concentration was 10.2 ± 1.9 mg L^{-1} during the period 2000-2004 at which indicates that this aquifer is suffering severe nutrient pollution (Iribar et al., 2007). It is well-established that excessive nutrients passing through surface- and ground-waters have harmful consequences on ecosystem structure and functioning (Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999). At a time of increasing concerns about the impact of water quality on aquatic life and on the sustainability of water resources, recent works support that biological remediation (i.e. pollutant removal by biological activity such as microbial metabolisms) by benthic biofilms appears as a promising way to cope with eutrophication threats (Singh et al., 2006; Sun et al., 2009; Cao et al., 2012).

Indeed, biofilms, especially phototrophic biofilm, can act as a sink for nutrients in the water column, strengthen vertical and horizontal connectivity of resources and play a role in the self-depuration processes of river waters (Pusch et al., 1998; Sabater et al., 2002; Battin et al., 2003; Teissier et al., 2007). Such biofilm depuration function is the key fundament of this thesis.

Pathways for ecosystem nitrate removal are complicated and hard to be exhaustively listed. A general diagram, from Burgin et al. (2007) is shown in Fig. 1-1. Nitrate assimilation into algal or bacterial biomass (producing organic N) and respiratory denitrification by bacteria (producing N_2) are thoroughly examined pathways. Dissimilatory nitrate reduction to ammonium (DNRA) is one of the alternatives to respiratory denitrification e.g. involving fermentation and sulphur oxidation. Besides, iron-driven denitrification (iron oxidation, producing NO_2^-) and anaerobic ammonium oxidation (producing N_2) are two other important, but less known pathways. Microorganisms can participate actively in these pathways. Among

these nitrate reduction pathways, denitrification is the main process which reduces nitrogen loading from rivers entering marine ecosystems (Galloway et al., 2003). Denitrifying bacteria are present and active both in the phototrophic biofilms and hyporheic zone heterotrophic biofilms (Pinay et al., 2009; Lyautey et al., 2013). Iribar et al. (2007) studied the attached and free-living bacterial communities involved in the process of denitrification in Garonne River, and suggested that bacterial denitrifying capability was present in the sediment-attached communities but not in those freely living in the groundwater.

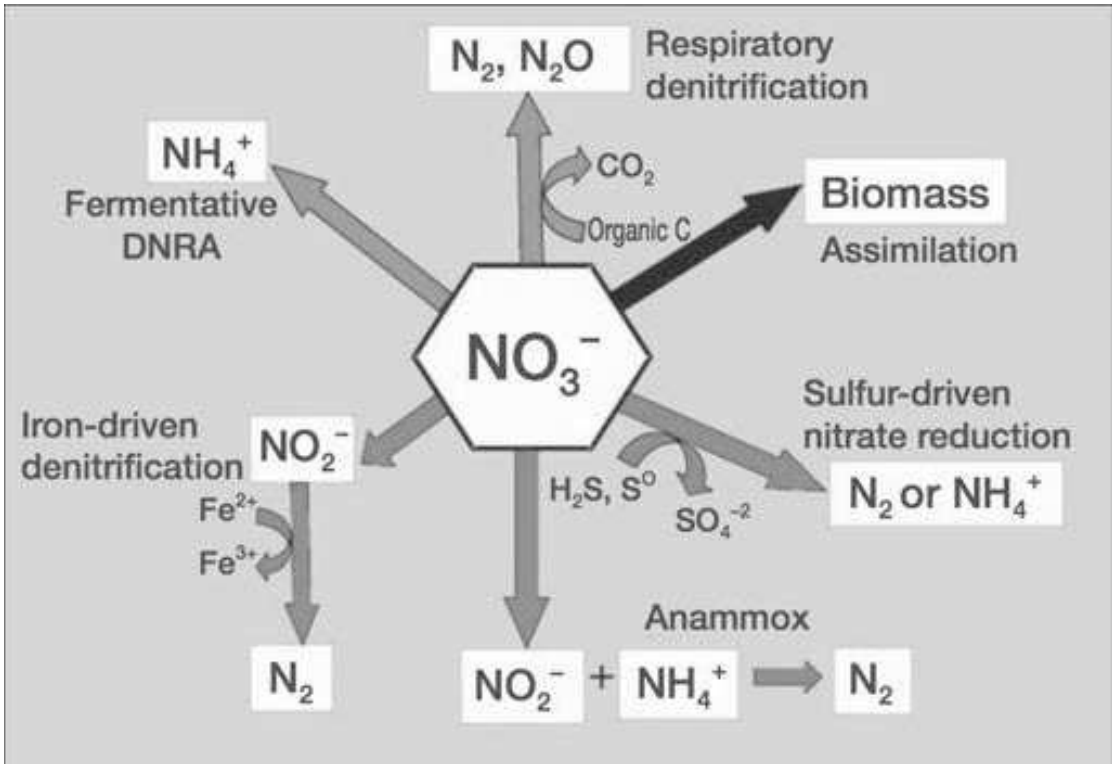

Figure 1-1 A general diagram of the nitrate removal pathways. Blue arrows denote autotrophic pathways, while purple arrows denote heterotrophic pathways (Burgin & Hamilton, 2007)

In hyporheic zones, toxic organic pollutants (e.g. herbicides) are accumulative due to the large surface area of sediments (Hedges & Keil, 1995; Pereira et al., 1996; Devault et al., 2009). Diuron (N-[3,4-dichlorophenyl]- N,N-dimethylurea; CAS No. 330-54-1) is a broad-spectrum residual herbicide. The diuron concentrations in European rivers reported in literature range between 2.1 - 36 μg L^{-1} (López-Doval et al., 2009). Diuron can inhibit photosynthesis in algae and cyanobacteria by limiting the production of adenosine triphosphate (ATP) used for various metabolic processes (Corbett, 1984; Hayes, 1991; Pesce et al., 2006; Ricart et al., 2009). Since the hyporheic biofilm is non-photosynthetic (i.e. heterotrophic), it might not be affected by diuron (Proia et al., 2011). However, the microbial metabolisms that occurs in this biogenic structure might be able to interact with herbicide

molecules (e.g. diuron), and could thus play a role in the diuron removal process (Pesce et al., 2009; Vercraene-Eairmal et al., 2010; Pesce et al., 2013).

1.2.2 Meiofauna

1.2.2.1 Definition

The term "meiofauna" was introduced by Mare (1942). The size range used for defining meiofauna somewhat varies depending on the authors e.g. size range of 44-500 µm after Higgin and Thiel (1988), 42-500 and 63µm-1000 µm after Giere (2009). Nowadays, members of meiofauna are considered mobile and occasionally haptosessile benthic invertebrates, smaller than macrofauna but larger than microfauna (Giere, 2009). Some authors proposed that large protozoans (ciliates, amoebozoans) fall in the range of the body size can be considered as meiofaunal organisms (Giere, 2009). In addition to the "permanent" meiofauna, some "temporary" meiofauna organisms belong to the meiofauna size range during early stages but later become macrofauna (e.g. insect larvae). Fig. 1-2 shows the principal examples of the main meiofaunal groups observed in phototrophic biofilms of Garonne River, including nematodes, rotifers and chironomid larvae. Meiofauna are abundant in all ecosystems e.g. soil, freshwater and marine (Murphy, 1958; Gerlach, 1971; Rundle et al., 2002). Their presence in biofilms seems also ubiquitous (Gaudes et al., 2006; Kathol et al., 2011; Majdi et al., 2012a; Carpentier et al., 2014).

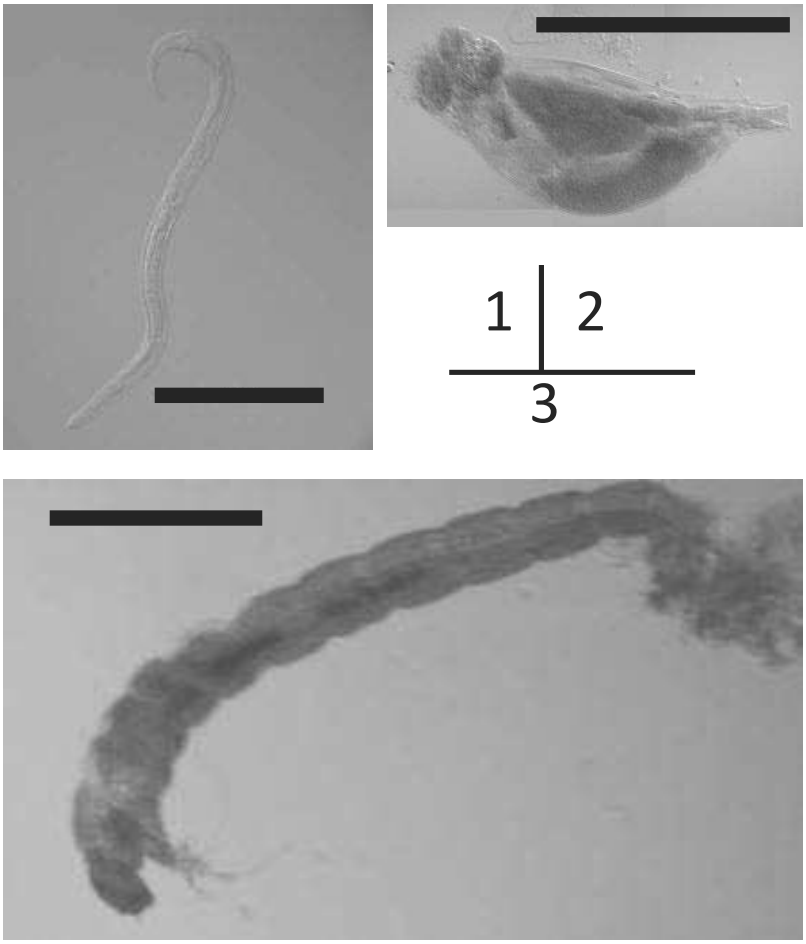

Figure 1-2 Examples of three main groups of meiofauna observed in the epilithic biofilm from the Garonne River: 1) nematode (*Chromadorina* sp.), 2) rotifer (*Proales* sp.), 3) Chironomid larvae. Black bars represent 200 µm. Photos were taken by B. Mialet, N. Majdi and F. Azémar from Ecolab UPS Toulouse.

1.2.2.2 Biofilm-associated meiofauna

Many ecological researches have studied invertebrates in biofilms. For instance, Hillebrand (2009) examined 835 studies on the grazing activity of macrofauna in phototrophic biofilms (i.e. periphyton). However, it is surprising that relatively little attention has been paid to the meiofauna dwelling in biofilms. The abundance of meiofaunal nematodes can reach up to 10^5-10^6 ind. m^{-2} in cyanobacterial biofilms (Farmer, 1992; Gaudes et al., 2006) and 3.19×10^6 ind. m^{-2} in diatom dominated biofilms (Majdi et al., 2012a). Their colonization of biofilms are taxon-specific, for example, during the periods after floods, rotifers are faster colonizers than nematodes in the epilithic Garonne River biofilms, and their density is closely coupled to biofilm biomass (Majdi et al., 2012a). Besides, the meiobenthic rotifers Bdelloidea have pedal adhesive glands that secrete a sticky cement used for temporary attachment to the substrate (Ricci & Balsamo, 2000) which allows them to have high resilience to flood disturbance (Majdi et al., 2012a). The diversity of biofilm-associated

meiofauna is habitat specific. For example, in Garonne River biofilms, nematodes and rotifers are the dominated taxa followed by chironomids, oligochaetes, harpacticoid copepods and tardigrades (Majdi et al., 2012a), while in the biofilm from Brouage coastal mudflat (France), main meiofaunal taxa are nematodes, copepods, foraminifers, ostracods and plathelminths (Carpentier et al., 2014). and in a second-order stream of Lower Austria, harpacticoids, ostracods and nematodes are found to be the main meiofaunal groups (Schmid Araya, 2000). Although the grazing activity of nematodes could not induce evident decrease of biofilm biomass (Majdi et al., 2012b; 2012c), Mialet et al. (2013) analyzed *in situ* pigment contents of bdelloid rotifers of the Garonne River, and found that rotifers could daily remove a substantial fraction (up to 28 %) of cyanobacterial biomass, showing an interesting selective feeding behavior of rotifers.

1.2.2.3 Role of meiofauna in the N cycle

Table 1-1 Summary of previous literature on meiofauna effect on nitrate.

Dominant meiofaunal taxa	Habitats	Effects on nitrate concentration	Observed processes	References
Nematodes	Marine, sediment	-	Denitrification	Hentschel et al. 1999
Copepods	Marine, sediment	+	Nitration	Parent et al. 1999
Nematodes	Marine, sediment	-	Denitrification	Bonaglia et al. 2014
Copepods	Marine, sediment	-	Dissimilatory	Stock et al. 2014

Several authors have studied the responses of meiofauna to the context of excessive nutrient concentrations. Hillebrand et al. (2002) observed no significant effect of nutrient amendments on meiofaunal biomass in sediments from either freshwater lake or coast. Saltmarsh meiofaunal density responded inconsistently and variably to long-term (5 years) nutrient enrichment in marine muddy sediments (Mitwally & Fleeger, 2013). In contrast, meiofauna (biomass or density) in sediments was positively affected 1) by fertilization in streams (Gaudes et al., 2012) and 2) by nutrient addition in microcosms (Ristau et al., 2013). As such, the nutrient effect on biofilm-associated meiofauna is still unclear.

Besides the effect of nutrient concentrations on meiofauna, another question is whether meiofauna have a role in nutrient cycling? Most studies dealing with the effects of fauna on benthic biogeochemistry e.g. the N cycle, have considered large animals because

they are easy to manipulate in the laboratory and are expected to physically alter microbial pathways through bioturbation. Benthic macrofauna (invertebrates size > 1 mm) is widely recognized to play an important role in the regulation of carbon mineralization, nutrient regeneration and coupled nitrification/denitrification (Aller, 1994; Lillebø et al., 1999; Gerino et al., 2003; Gilbert et al., 2003; Welsh, 2003). Macrofaunal activity is known to either enhance denitrification due to particle reworking and burrowing, ventilation and bioirrigation (Karlson et al., 2007; Stief, 2013), or have negative impact on denitrification by means of dissimilatory nitrate reduction to ammonium (Bonaglia et al., 2013). However, very few studies concern the effects of meiofauna on nutrient cycle e.g. N fluxes. To the best of our knowledge, other than our work, only four papers (summarized in Table 1-1) treated this aspect.

Parent et al. (1999) found that, in marine sediments, copepod-dominated meiofaunal biomasses less than 0.16 g m^{-2} increase the nitrification rate two to five times. They explained this by the fact that when present in low biomass meiofauna feeds only on heterotrophic bacteria, but that they feed on both heterotrophs and nitrifiers when present in higher biomass. Besides, Hentschel et al. (1999) showed that nematodes of the family Stilbonematinae are known for their highly specific association with ectosymbiotic bacteria which are capable of denitrification.

Recently, Bonaglia et al. (2014) examined the effects of low and high density of meiofauna in the presence or absence of macrofauna on element cycling in marine sediments, and found that meiofaunal bioturbation activity has a stimulating effect on nitrifying and denitrifying bacteria (Fig. 1-3). It is suggested that this enhanced dinitrogen production is not due to direct respiration of nitrate by the meiofauna, as it has been shown for some species of foraminifera, a common unicellular meiofauna group (Risgaard-Petersen et al., 2006; Hogslund et al., 2008), but due to the high meiofauna bioturbation.

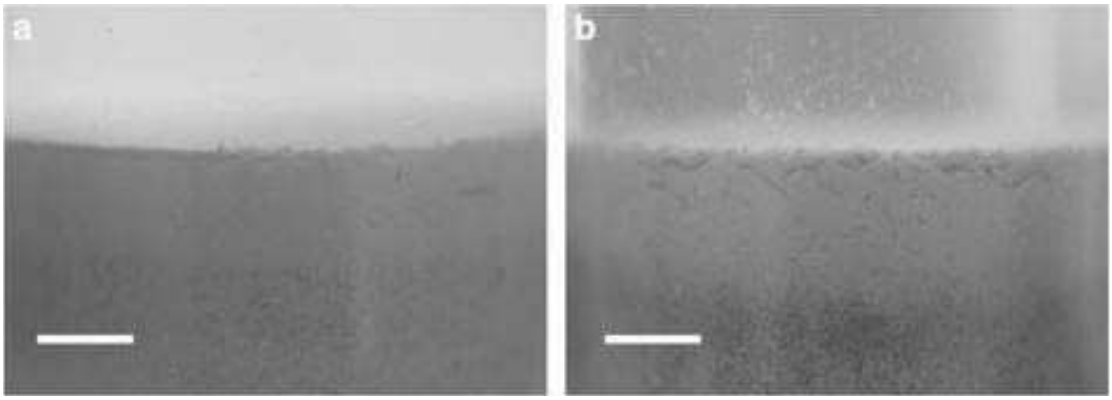

Figure 1-3 Digital camera pictures by Bonaglia (2014) showing the different meio-bioturbation intensity in the upper sediment layer of microcosms (marine sediment cores) from a low meiofauna density treatment (a) and the high meiofauna density treatment (b). Length of scale bars is 500 µm.

Another recent study evaluated the interactions between benthic copepods, bacteria and diatoms in intertidal sediments (Stock et al., 2014). Copepods could enhance DNRA over denitrification. It is hypothesized that this is an indirect effect, by providing extra carbon for the bacterial community through the copepods' excretion products. However, Stock et al., (2014) did not observe an actual nitrate reduction, suggesting that the denitrification activity reduced by copepods was compensated by another nitrate reducing process (Fig. 1-4).

In summary, the interactions between meiofauna and microorganisms could considerably contribute to the N cycle in aquatic ecosystems.

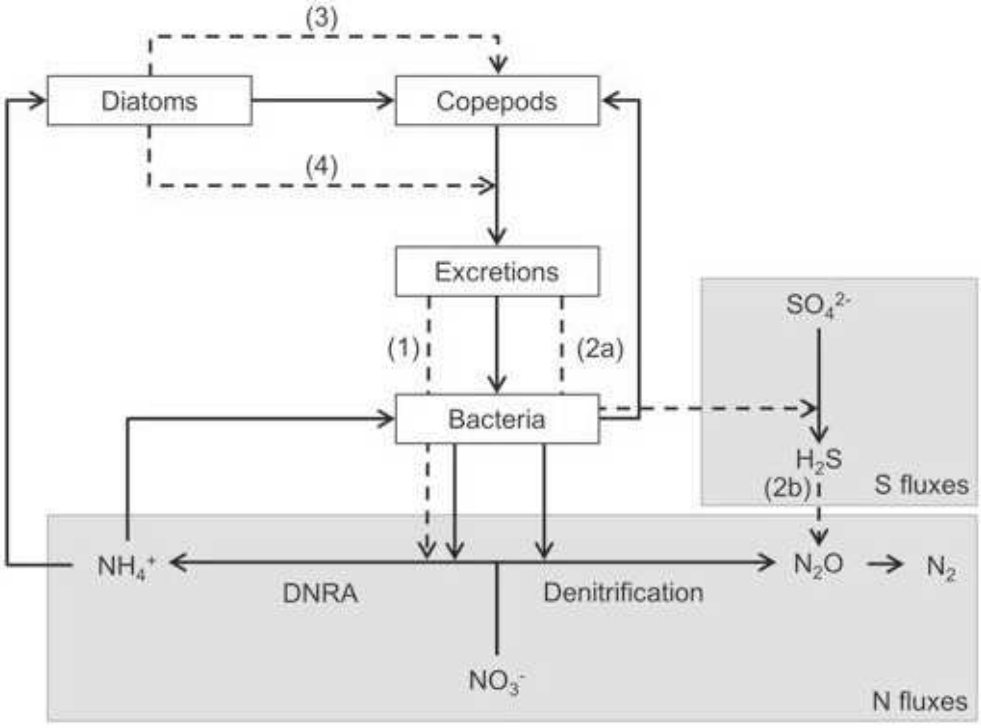

Figure 1-4 Summary of the assumed interactions between copepods, bacteria and diatoms by Stock et al. (2014). The assumed interactions which affect denitrification are indicated with dashed arrows. Bacteria mediated relevant reduction reactions of the nitrogen pathway and sulfur pathway are enclosed by grey boxes indicated with respectively 'N fluxes' and 'S fluxes'. Meiobenthic copepods feed on both diatoms and bacteria, and produce excretion products (excretions). This excretion stimulates bacterial

growth. Bacterial community is involved in the reduction of SO_4^{2-} to H_2S, and, in the reduction of NO_3^- to NH_4^+ (DNRA) and N_2O+N_2 (denitrification). The produced NH4+ is assimilated by both bacteria and diatoms. Copepods affect the N_2O production rate through their excretion products which provide an extra carbon source stimulating mainly the DNRA bacteria activity (1) and also enhancing sulfate reduction (2a), which results in more H_2S. The increased H_2S inhibits denitrification (2b). Diatoms have no direct effect on the N_2O production rate, but do have an indirect effect by enhancing the survival of the copepods (3) and influencing the quantity and composition of the copepods' excretion products (4).

1.2.3 Objectives and organization of the thesis

In this context, this thesis research tested the hypothesis that, the activity (feeding and bioturbation) of biofilm-associated meiofauna is a factor positively influencing biofilm functioning in relation to river water quality, especially concerning the N- cycling. The thesis focused not only on phototrophic biofilms growing on riverbeds, but also on heterotrophic biofilms growing in hyporheic zones since hyporheic meiofauna can be abundant (Schmid Araya, 2000; Schmid Araya & Schmid, 2000). Besides, to fully understand the role of biofilm-associated meiofauna in the complex lotic ecosystem, in the latter system, macrofauna was also taken into account, which allowed to test the hypothesis that uptake of nutrients by sediment communities is more effective when the diversity of the community increases.

To understand the role of biofilm-dwelling meiofauna in the N cycle within both benthic systems and hyporheic zones, four experiments were conducted in laboratory.

The first experiment (Chapter 2) was the first step of this thesis work which aimed to test whether biofilm-associated meiofauna can respond at short-term to nutrient input in relation with the dynamics of biofilm nitrate uptake rates. This experiment used a phototrophic epilithic biofilm which was grown in the field.

Since we observed a response of meiofauna to nitrate enrichment and the results suggested a possible link between bacteria-meiofauna interactions and short-term N uptake capacity of biofilms, the second experiment (Chapter 3) focused on the effect of meiofauna density on phototrophic biofilm N uptake in lotic condition microcosms.

The meiofauna dwelling in heterotrophic biofilms of the hyporheic zone was studied in Chapters 4 and 5. In Chapter 4, the role of meiofauna on N-uptake in heterotrophic biofilms was tested in microcosms. Additionally, since micro-organisms which mediate nutrient cycling and organic matter transformation are under a top-down control by organisms of higher trophic levels, this experiment as a whole examine the role of cross-communities (biofilm, meiofauna, macrofauna) diversity on the uptake of nitrate and dissolved organic carbon.

The fourth experiment (Chapter 5) was designed under the same context as the experiment in Chapter 4, however treatments with an herbicide (diuron) were added. The objective was to test whether invertebrates can modify the potential perturbation caused by diuron on the biofilm N uptake. While I entirely performed the experiments described in Chapters 2 and 3, my contribution to experiments of Chapters 4 and 5 was focused on data analyses and redaction of the manuscripts.

Chapter 2: Effets à court terme d'un enrichissement en nutriments sur un biofilm épilithique : taux de consommation de $N\text{-}NO_3$ et réponse de la méiofaune associée

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2.1 Résumé de l'article

2.1.1 Contexte et objectifs

Les apports excessifs en nutriments sont nuisibles pour le fonctionnement des écosystèmes aquatiques (Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999). Les biofilms phototrophes puisent les nutriments dans la colonne d'eau. Ils renforcent ainsi les échanges verticaux et horizontaux des ressources et contribuent aux processus « d'auto-épuration » des écosystèmes lotiques (Pusch et al., 1998; Sabater et al., 2002; Battin et al., 2003; Teissier et al., 2007). La capacité de consommation des nutriments via leur incorporation dans la biomasse de ces biofilms, peut être très élevée puisque les nutriments sont intensivement recyclés par les communautés benthiques (Bernot & Dodds, 2005). De plus, les processus bactériens tels que la dénitrification par exemple (respiration des nitrates aboutissant à la production de diazote), contribuent à la consommation apparente des nutriments dans les cours d'eau (Bernot & Dodds, 2005; Ribot et al., 2013).

Les études ayant examiné l'impact de la méiofaune sur la micro-architecture, la porosité et les processus biogéochimiques des biofilms, ont montré que la méiofaune peut (1) stimuler la croissance des bactéries et des processus associés de minéralisation, notamment, par leur capacité à sécréter du mucus qui retient les particules détritiques, et/ou, grâce à des capacités protéolytiques (e.g. Riemann & Helmke, 2002; Nascimento & Naslund, 2012) ; (2) augmenter la production primaire et favoriser la circulation de l'oxygène dans les biofilms phototrophes (Mathieu et al. 2007) et (3) probablement modifier la pénétration de la lumière et la circulation des solutés dans les sédiments superficiels (e.g. Pinckney et al., 2003). Donc, il est très probable que la méiofaune et les micro-organismes interagissent dans ces biofilms. Dans ce contexte, l'hypothèse d'une influence indirecte de la méiofaune sur le fonctionnement du biofilm et notamment sur les processus liés à la consommation des nutriments est émise. Dans un premier temps, cette étude vise à examiner la réponse potentielle de la méiofaune associée aux biofilms face à un enrichissement du milieu en nutriments.

2.1.2 Principaux résultats et discussion

Cette étude examine l'effet d'un enrichissement en nutriments sur un biofilm phototrophe de rivière, préalablement cultivé en milieu naturel (Garonne, France), par le suivi simultané du taux consommation du $N\text{-}NO_3$ par le biofilm et de la méiofaune associée, en microcosmes exposés pendant 5 jours à différentes conditions de concentrations en

nutriments (e.g. eau de la Garonne non enrichie et enrichie en nutriments). A la fin de la période expérimentale, la communauté méiobenthique était largement dominée par les nématodes et rotifères, en terme de densité. Une augmentation significative de la densité et biomasse de la méiofaune et en particulier des rotifères, a été observée dans les microcosmes enrichis en nutriments comparés à ceux contenant de l'eau naturelle non enrichie. La densité bactérienne associée aux biofilms était aussi significativement plus élevée dans les microcosmes enrichis bien que cela n'ait pas eu d'effet sur la biomasse totale des biofilms. Par contraste, aucune modification concernant la biomasse microalgale n'a été observée par l'analyse *HPLC* du contenu pigmentaire des biofilms (concentration en chlorophylle *a* et en pigments biomarqueurs).

Le taux de consommation de $N\text{-}NO_3$ moyen par les biofilms était significativement plus élevé dans les microcosmes enrichis, durant toute la période d'étude. Ce taux de consommation a atteint un plateau approximant 104 µg g-1 AFDM h^{-1}, pour des concentrations en $N\text{-}NO_3$ relativement faibles (de 0,2 à 0,6 mg L^{-1}). Lorsque les concentrations ont dépassé ce seuil, le taux de consommation s'est accru de façon linéaire en fonction des concentrations en $N\text{-}NO_3$ utilisées.

Le résultat le plus remarquable concerne l'augmentation significative et rapide de la densité et biomasse des rotifères dans les microcosmes enrichis. Il est envisageable que cette augmentation soit le résultat d'un effet indirect lié à une stimulation de la croissance des ressources trophiques microbiennes (probablement bactérienne et microalgale) de la méiofaune. D'autre part, les rotifères pourraient avoir eux-mêmes contribué à stimuler la croissance des bactéries par leur activité de bioturbation qui pourrait améliorer la circulation de l'oxygène et les échanges de solutés comme cela a été montré au préalable pour les nématodes (Traunspurger et al., 1997; Riemann & Helmke, 2002; Teissier et al., 2007; Nascimento & Naslund, 2012). Cette suggestion est confortée par l'accroissement concomitant des densités de rotifères et de bactéries. L'augmentation de la densité des rotifères en réponse à l'enrichissement en nutriments des microcosmes pourrait avoir, par un processus de feedback, augmenté la croissance bactérienne et donc par conséquent, la consommation des nutriments par les biofilms. Dans l'ensemble cette étude suggère que les interactions méiofaune-micro-organismes peuvent indirectement favoriser, la capacité des biofilms phototrophes à améliorer la qualité de l'eau.

Short-term effects of nutrient enrichment on river biofilm: N–NO_3^- uptake rate and response of meiofauna

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2.2 Abstract

Biofilms play a key role in self-depuration processes in rivers. While meiofauna is known to be abundant within river phototrophic biofilms and to perform both grazing and bioturbation within these matrixes, it is still unknown whether the activity of biofilm-associated meiofauna can influence the ability of biofilms to improve river water quality. In this study, we explored the effects of nutrient enrichment on river biofilm N-NO_3^- uptake rates and associated meiofauna in microcosms for 5 days under nutrient enriched/non-enriched conditions. Short-time nutrient enrichment stimulated biofilm-associated bacterial and rotifer density, as well as the biofilm uptake rates of N-NO_3^-, but not algal biomass. Under non-enriched conditions, N-NO_3^- uptake rate tended to saturate around 104.2 μg g^{-1} AFDM h^{-1}. At higher N-NO_3^- concentrations, realised under enrichment, N-NO_3^- uptake rate seemed to increase linearly, reaching up to 439.2 μg g^{-1} AFDM h^{-1}. Our results showed a rapid response of rotifers to nitrate enrichment and suggest a possible link between bacteria-meiofauna interactions and the short-term N uptake capacity of biofilms.

Keywords:

Self-purifying capacity, nitrate retention, biofilm, meiobenthic rotifers, streams

2.3 Introduction

It is well-established that excessive nutrients concentrations, e.g. of nitrate and phosphate passing through surface- and ground-waters, have harmful consequences on ecosystem structure and functioning (Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999). At a time of increasing concerns about the impact of water quality on aquatic life and on the sustainability of water resources, recent works support that biological remediation (i.e. pollutant removal by microbial metabolism) by benthic biofilms appears as a promising way to cope with eutrophication threats (Singh et al., 2006; Sun et al., 2009; Cao et al., 2012).

In running waters, biofilms growing on hard submerged substrate are complex assemblages of microalgae, protozoans, fungi, bacteria and small invertebrates clustered within a self-produced mucous matrix of exopolymeric substances (Lock et al., 1984; Costerton et al., 1995). Biofilms act as a sink for nutrients in the water column, strengthen vertical and horizontal connectivity of resources, and play a role in the self-depuration processes of running-waters (Pusch et al., 1998; Sabater et al., 2002; Battin et al., 2003; Teissier et al., 2007). Short-term retention of nutrients via assimilatory uptake (i.e. incorporation of compounds in the biomass) in biofilms can be very high as nutrients are intensively recycled within benthic communities (Bernot & Dodds, 2005). Furthermore, specific bacterial processes such as nitrification (i.e. oxidization of NH_4^+ to NO_3^-) and denitrification (i.e. respiratory process reducing NO_3^- to N_2), contribute to apparent uptake of nutrients in streams (Bernot & Dodds, 2005; Ribot et al., 2013).

Meiofauna (i.e. benthic invertebrates passing through a 500 µm mesh sieve and retained on 50 µm meshes, Giere, 2009) are extremely abundant in epilithic river biofilms (Gaudes et al., 2006; Majdi et al., 2012a). Although their grazing pressure on biofilm microphytobenthos is rather low (Majdi et al., 2012b; 2012c; Mialet et al., 2013), their activity within the mat can affect oxygen turnover (Teissier et al., 2007) and seemingly other key processes such as biofilm detachment and the release of secondary metabolites in the water column (Sabater et al., 2003; Gaudes et al., 2006; Teissier et al., 2007). Recently, Derlon et al. (2013) have shown that, in gravity-driven membrane filtration water depuration systems, the presence of nematodes and oligochaetes increases the heterogeneity and porosity of membrane-associated microbial biofilms, and consequently increases the efficacy of filtration process used to treat organically polluted waters. Riemann & Helmke (2002) report that locomotion of nematodes creates dense micro-burrows through agar plate matrixes.

Studies examining the impact of meiofauna on the microarchitecture, porosity and biogeochemical activity of biofilm have shown that meiofauna can: (1) stimulate the growth of bacteria and associated mineralization processes e.g. through agglutination of detritus particles by mucus secretions or proteolytic capacity (e.g. Riemann & Helmke, 2002; Nascimento & Naslund, 2012); (2) enhance the primary productivity and oxygen turn-over of diatom biofilms (Teissier et al., 2007) and (3) likely modify light penetration and increase solute transport rates in superficial sediments (e.g. Pinckney et al., 2003). Thus, it is likely that positive interactions between meiofauna and micro-organisms occur in epilithic biofilms. Since microphytobenthos and bacteria are key organisms involved in organic and inorganic nutrient retention processes in biofilms (Sabater et al., 2002; Cardinale, 2011), it can be expected that the interactions between meiofauna and micro-organisms stimulate the self-depuration processes associated with biofilms in natural running waters.

Human activities can modify nutrient concentrations in streams, sometimes on short timescales, for example pulses caused by agricultural runoff during high rainfall periods (Lassaletta et al., 2009). Stream biofilms can adapt their uptake rate of nutrients according to nutrient availability and speciation in the environment (Dodds, 2003; Bernot & Dodds, 2005; Ribot et al., 2013), generally following a Michaelis –Menten response (but see discussion) (Payn et al., 2005; Earl et al., 2006; Covino et al., 2010; O'Brien & Dodds, 2010). Moreover, recent studies report that nutrient enrichment can induce increase in the density of marine and freshwater sediment-dwelling meiofauna, although the observed functional responses are slow and highly variable (e.g. Hillebrand et al., 2002; Posey et al., 2002; Mitwally & Fleeger, 2013; Ristau et al., 2013). As detailed above, since it has been shown that meiofauna can influence primary productivity and stimulate bacterial growth, we hypothesized that biofilm-dwelling meiofauna could indirectly influence biofilm functions related to nutrient uptake. As the first step, this study aims to examine the short-term response of biofilm-dwelling meiofauna and microbial communities to nitrate enrichment in relation with the dynamics of the biofilm uptake rates of nitrates.

2.4 Methods

2.4.1 *In situ* biofilm growth

We wedged a total of 36 rubber stoppers (upper surface area = 12.56 cm^2) onto the Garonne river bed at 30 km upstream Toulouse (location: 01°17'50"E, 43°23'43"N; elevation: 175 m asl). The Garonne River catchment is the third largest in France (~57,000 km^2). This

catchment is mostly agricultural, containing only one major urban area: Toulouse (> 1 million inhabitants). At this site, nutrient conditions are oligotrophic (Lyautey et al., 2003; Muylaert et al., 2009), and a shallow river bed together with a low shading by riparian vegetation usually allows a thick epilithic phototrophic biofilm, crowded with meiofauna, to coat any hard submerged substrates (Majdi et al., 2012a). Biofilm colonization of rubber stoppers was allowed during 56 days (20th September–15th November 2012), a period deemed long enough for the establishment of mature biofilm communities in temperate rivers (e.g. Norf et al., 2009). The ambient N-NO_3^- concentration at the studied site – measured from water river samples collected for non-nutrient enriched microcosms (see below) – ranged between 0.48 and 0.55 mg l^{-1} during the biofilm incubation.

At the end of the colonization period, the rubber stoppers covered by biofilm were retrieved and immediately placed in polyethylene microcosms (Ø52 mm, h 68 mm) filled with 100 ml river water. Meanwhile, 40 l of river water were sampled. Microcosms and water were transported to the laboratory in cool boxes within 2 h, with minimal disturbance.

2.4.2 Experimental design

The experimental design consisted in two biofilm conditions (with biofilm: BIOF and without biofilm: WAT) crossed with two nutrient availability conditions (NAT and NUT, see above) (Fig. 2-1). So, our experiment had four treatments: (1) clean rubber stopper incubated with non-enriched Garonne water (NAT-WAT, n = 6), (2) clean rubber stopper incubated with nutrient enriched water (NUT-WAT, n = 6), (3) rubber stopper covered by biofilm incubated with non-enriched Garonne water (NAT-BIOF, n = 12), (4) rubber stopper covered by biofilm incubated with nutrient-enriched water (NUT-BIOF, n = 12). To avoid high variability of biofilm samples, the numbers of BIOF treatments were doubled than that of WAT treatments. All biofilm microcosms were incubated for 5 days under the same experimental controlled conditions (see above).

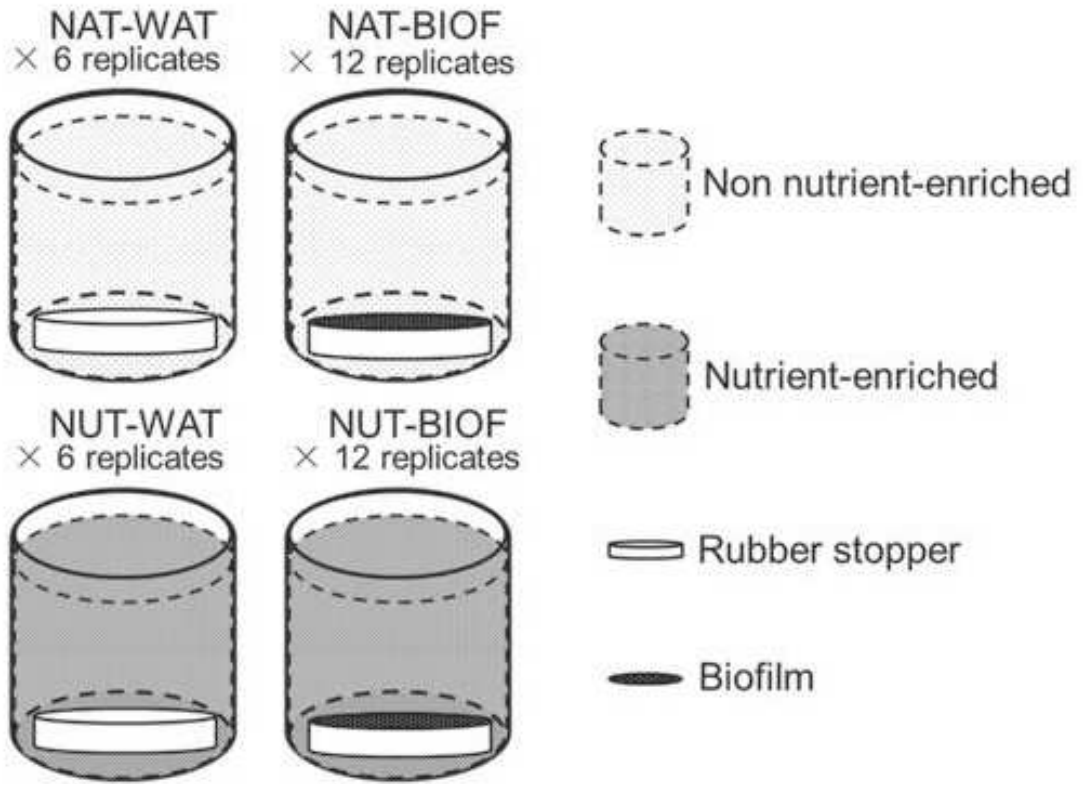


Fig. 2-1 The scheme of the experimental design

By monitoring the Garonne River, Leflaive et al. (2008) show that at our sampling site, total P and total N concentrations vary over a year (February/2005 to February 2006) from 0.01 to 0.05 and 0.4 to 1.4 mg/L, respectively, which corresponds to oligotrophic conditions (Wetzel, 2001). We used GF/C filtered river water as a non-nutrient enriched treatment (NAT). For the nutrient-enriched treatment (NUT), we added KNO_3 (10 mg l^{-1}, i.e. NO_3^-, 6.14 mg l^{-1}), Na_2HPO_4 (1 mg l^{-1}) and $CH_3COONa{\cdot}3H_2O$ (30 mg l^{-1}) to GF/C filtered river water in order to mimic downstream eutrophic condition as indicated in previous study (DIN > 2 mg l^{-1} and SRP > 20 μg l^{-1}, Muylaert et al., 2009) i.e. at the beginning of the experiment, in NAT treatment: $N\text{-}NO_3^-$, 0.54 ± 0.002 mg l^{-1}; $P\text{-}PO_4^{3-}$, 14 ± 0.8 μg l^{-1}, in NUT treatments: $N\text{-}NO_3^-$, 2.03 ± 0.004 mg l^{-1}; $P\text{-}PO_4^{3-}$, 401 ± 3.4 μg l^{-1} (n = 6, se). High phosphate and acetate (as carbon substrate) addition into the nutrient-enriched treatment was used to prevent microbial growth limitation in microcosms during the experiment.

In the laboratory, the bottom and sides of the rubber stoppers were carefully scrubbed (to restrain biofilm only to upper surfaces), and the stoppers were quickly transferred into new microcosms filled with 100 ml GF/C filtered Garonne water. Microcosms were acclimatized during 24 h to experimental controlled conditions: i.e. 10°C; light:dark 12 h:12 h, 2300 lm m^{-2}. After the 24 h acclimatization, the water in the microcosms was carefully

removed and replaced by non-enriched Garonne water (NAT treatment: 18 microcosms) or nutrient-enriched (NUT treatment: 18 microcosms).

2.4.3 Sample treatment

One ml of water was sampled daily (t = 4 h, 24 h, 48 h, 72 h, 96 h, 120 h) from each microcosm and filtered (0.22 µm PTFE syringe filter) prior to analysis of Cl^- and N-NO_3^- concentrations by high-performance ionic chromatography (Dionex DX-120, Thermo Fisher Scientific Inc., Waltham, MA, USA) following standard procedures (NF EN ISO 10304-1, 1995). For each biofilm treatment, meiofauna density and biomass as well as density of bacteria and pigment concentrations were determined at the end of the experimental period (t = 120 h). The biofilm covering stoppers was gathered from each microcosm and divided into 3 subsamples for the following measurements:

(1) For meiofaunal density and biomass quantification, half of the total biofilm surface on stoppers (i.e. 6.28 cm^2) was carefully scraped, and preserved in 10 ml formaldehyde solution (5 % final concentration) with addition of 100 µl of 1 % Rose Bengal stain. Meiofauna were counted in a Dolfuss cell (Elvetec Services, Clermont-Ferrand, France) under a stereomicroscope (9–90×). A number of individual nematodes (n = 21) and rotifers (n = 32) were photographed to measure their body dimensions using ImageJ software version 1.46r (Abràmoff et al., 2004). Mean individual dry mass was assessed from standard biometric conversions of the organism's body dimensions (Giere, 2009; Majdi et al., 2012a), and multiplied by their density in biofilms to estimate biomass data.

(2) For bacterial density measurement, a 200 µl subsample of the previous described homogenized 10 ml formaldehyde – fixed sample (containing biofilm and associated meiofauna) was used following a standard DAPI-staining method (Porter & Feig, 1980). A gentle sonication step was carried out to maximize bacterial detachment from algal aggregates prior counting (Buesing & Gessner, 2002). Bacterial counting was performed under a Leitz Dialux microscope (1250×) fitted for epifluorescence: HBO 100 W mercury light source (Osram, Winterthur, Switzerland), with an excitation filter for 270 and 450 nm, a barrier filter of 410 nm and a 515 nm cut-off filter. All density calculations of bacteria were referred to the corresponding scraped biofilm area.

(3) For the assessment of the algal community composition and biomass, a quarter of the total biofilm surface on stoppers (i.e. 3.14 cm^2) was scraped, pelletized (3220 g, 20 min) and freeze-dried to remove excess water. Biofilm pellets were weighed, and algal pigments from each obtained pellet were extracted (15 min at –20°C) in a total of 5 ml 98% cold-

buffered methanol with 2% of 1M ammonium acetate (Buffan-Dubau & Carman, 2000). Algal pigment release was favoured by ultra-sonication (Sonifier 250A, Branson Ultrasonics corp., Danbury, CT, USA). One ml of the pigment extract so obtained was then filtered on 0.2 µm PTFE syringe filter and analysed using a high-performance liquid chromatograph (HPLC) consisting of a 100 µl loop auto-sampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies inc., Santa Clara, CA, USA). The mobile phase was prepared and programmed according to the analytical gradient protocol described in Barlow et al. (1997). Pigment separation was performed through a C8, 5 µm column (MOS-2 HYPERSIL, Thermo Fisher Scientific Inc.). The diode array detector was set at 440 nm to detect carotenoids, and at 665 nm to detect chlorophylls and pheaopigments (Wright et al., 1991). Data analysis was performed using ChemStation software (version A.10.02, Agilent Technologies Inc.). Pigments were identified by comparing their retention time and absorption spectra with those of authentic standards (DHI LAB products, Hørsholm, Denmark). For pigment quantification, a response factor was calculated for each standard from the linear relationship between the concentration and the corresponding peak area on HPLC chromatograms.

(4) For the assessment of biofilm biomass, the remaining quarter of the total biofilm surface on stoppers (i.e. 3.14 cm^2) was scraped in aluminium cups, dried overnight at 55 °C, weighted for its dry mass (DM), then combusted during 8 h at 450 °C to determine its ash-free dry mass (AFDM).

2.4.4 Data analysis and statistics

Chloride (Cl^-) is recognized to be non-reactive in ecosystems (Schlesinger & Bernhardt, 2013). Thus, we used the changes in Cl^- concentrations during the experiment to calibrate N-NO_3^- concentrations against potential increase caused by both water evaporation and the previous samplings as Equation 1:

$$C_t' = C_0' \times (C_{t(Cl)} / C_{0(Cl)}), \qquad (1)$$

Where C_0' and C_t' (mg l^{-1}) are the N-NO_3^- concentrations before and after calibration at a given time (t in hours) respectively, $C_{0(Cl^-)}$ is the initial Cl^- concentration), $C_{t(Cl^-)}$ is the Cl^- concentration at a given time (t in hours).

We calculated the N-NO_3^- uptake rates measured in the water phase of the microcosms as U (µg h^{-1}) with Equation 2:

$$U = 0.1 \times (\Delta C_t / t), \qquad (2)$$

Where 0.1 is the volume of water in each microcosm in litre, ΔC_t is $N\text{-}NO_3^-$ concentration difference (in µg l^{-1}) between mean concentrations recorded in the water of the microcosms at 4 h (used as the concentration at the outset of the incubation) and the concentration recorded at a given time (t in hour). The $N\text{-}NO_3^-$ uptake rates calculated for the water of the microcosms with/without biofilm were called U_{BIOF} and U_{WAT} respectively.

We calculated the $N\text{-}NO_3^-$ specific uptake rates realized by the biofilm as $U_{biofilm}$ (µg g^{-1} AFDM h^{-1}) with Equation 3:

$$U_{biofilm} = (U_{BIOF} - U_{WAT}) / AFDM_{biofilm}, \qquad (3)$$

$AFDM_{biofilm}$ is the ash free dry mass of the biofilm in NAT/NUT-BIOF at the end of the experiment, which is assumed as a constant for the two treatments throughout the short-term experiment.

Differences in $U_{biofilm}$ of $N\text{-}NO_3^-$ and in meiofaunal density and biomass between treatments were analysed by t-test. Assumption of homoscedasticity was tested with Levene's test. Data failing to fulfil homoscedasticity were log-transformed. The correlations between the $U_{biofilm}$ of $N\text{-}NO_3^-$ in each treatment and time were tested with Spearman's rank. All model-fitting calculations and statistical tests were performed using R software version 3.0.2 (R Core Team, 2013).

2.5 Results

2.5.1 Biofilm-associated meiofaunal, microalgal and bacterial communities

At the end of the experimental period (Fig. 2-2), nematodes, rotifers and chironomidae larvae were found in the biofilm but the first two dominated the meiofaunal group. The density and biomass of rotifers were significantly (i.e. twofold) higher under nutrient-enriched (NUT) vs non-enriched (NAT) conditions ($p < 0.001$). Though no significant trend was recorded for nematodes ($p > 0.05$), the higher density and biomass of rotifers under nutrient-enriched conditions resulted in a significant increase of total meiofauna density in NUT samples ($p < 0.05$). Chironomidae larvae were found in low densities: 0.74 ± 0.22 and 1.07 ± 0.74 ind. cm–2 respectively under NAT and NUT conditions between which no significant differences were found ($p = 0.55$). Bacterial density was higher under nutrient-enriched conditions (Fig. 2-3a; $p < 0.05$). However, this did not globally influence the total biofilm biomass (Fig. 2-3b; $p > 0.05$). The presence of typical biomarker pigments for diatoms (i.e. chlorophyll c, fucoxanthin and diadinoxanthin) indicated that biofilm algal

communities were dominated by diatoms. Neither biomarker pigments nor chlorophyll a concentrations showed a significant change under nutrient-enriched conditions (Fig. 2-4).

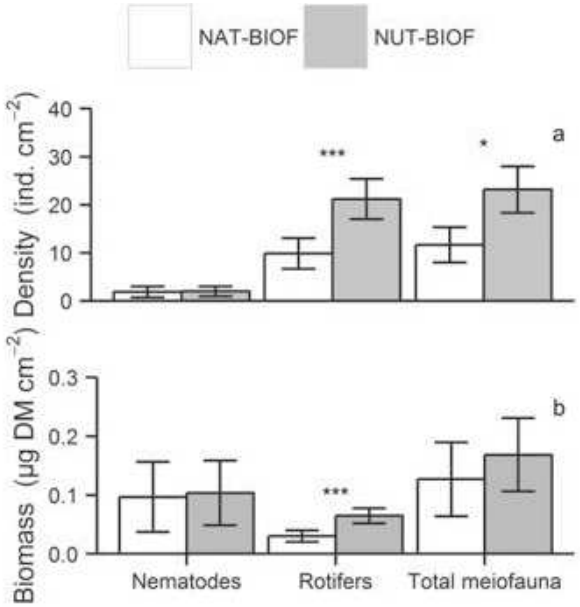


Fig. 2-2 Densities and biomass of nematodes, rotifers and total meiofauna (nematodes + rotifers) in NUT-BIOF (grey bars) and NAT-BIOF (white bars) at the end of the experiment. Values are mean ± SE (n = 12). Level of significance: * = p < 0.05, ** = p < 0.01 and *** = p < 0.001)

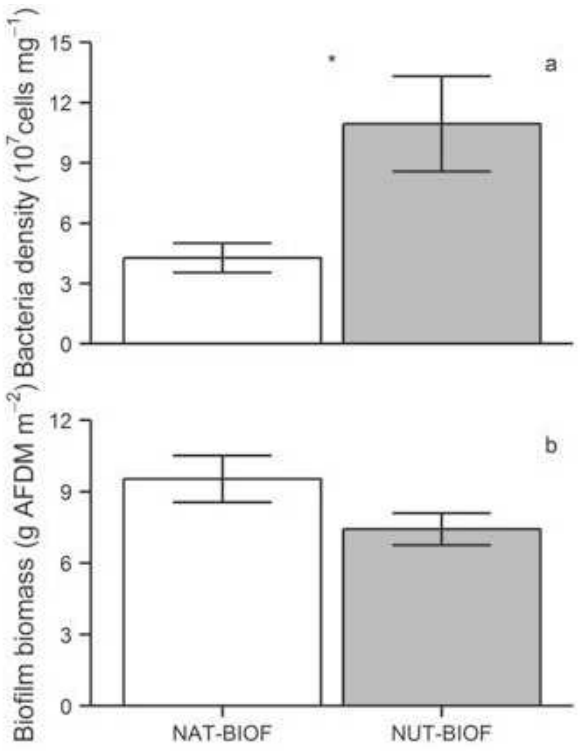


Fig. 2-3 Bacteria density in biofilm and biofilm biomass in NUT-BIOF (grey bars) and NAT-BIOF (white bars) at the end of the experiments. Values are mean ± SE (n = 12). Level of significance* = p < 0.05, ** = p < 0.01 and *** = p < 0.001)

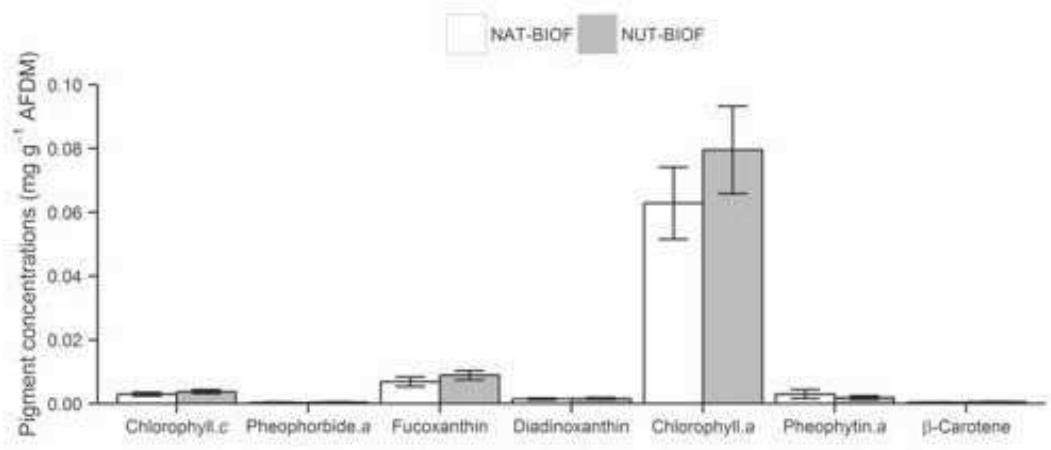


Fig. 2-4 Pigment concentrations in NUT-BIOF (grey bars) and NAT-BIOF (white bars) at the end of the experiments. Values are mean ± SE (n = 12)

2.5.2 N-NO_3^- biofilm uptake rates and kinetics

The $U_{biofilm}$ of N-NO_3^- was significantly higher in NUT-BIOF at all sampling occasions than in NAT-BIOF (Fig. 2-5, $p < 0.001$ for all dates*)*. $U_{biofilm}$ decreased significantly in NUT-BIOF and NAT-BIOF with increasing time after the start of the experiment, though the decrease was steepest in NUT-BIOF (Spearman's rank correlation; NAT-BIOF: $r = –0.65$, $p < 0.001$; NUT-BIOF: $r = –0.80$, $p < 0.001$).

The data from both treatments (NAT-BIOF and NUT-BIOF) were pooled in Fig. 2-6 and biofilm specific uptake rates values were related to N-NO_3^- concentrations measured in the microcosm water. The biofilm uptake rate values from each treatment (NAT-BIOF and NUT-BIOF) were very similar for similar N-NO_3^- concentrations (i.e. between 0.2 and 0.6 mg l^{-1}*)* occurring in both the NAT-BIOF and NUT-BIOF in the course of the experiment. Our results suggest that uptake ability of the biofilm reached a plateau (around 104.2 µg g^{-1} AFDM h^{-1}) under low nutrient concentrations, i.e. between 0.2 and 0.6 mg l^{-1} (Fig. 2-6). However, when exceeding this threshold, biofilm specific uptake rates seemed to increase linearly and consistently within the concentration range met in our experiment.

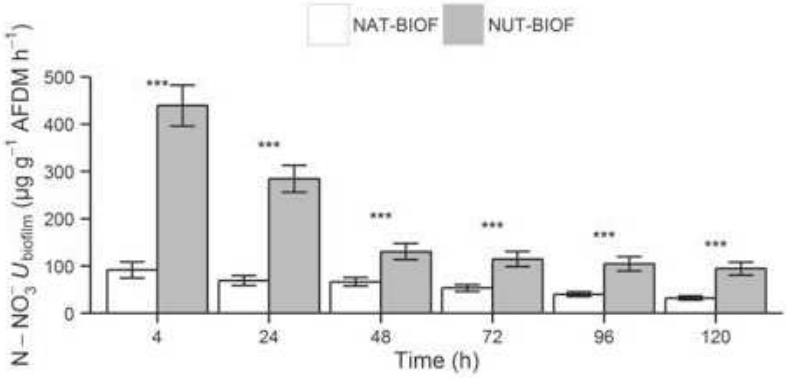


Fig. 2-5 $U_{biofilm}$ of N-NO_3^- in NUT-BIOF (grey bars) and NAT-BIOF (white bars) during the experimental period. Values are mean ± SE (n = 12). Level of significance: * = p < 0.05, ** = p < 0.01 and *** = p < 0.001)

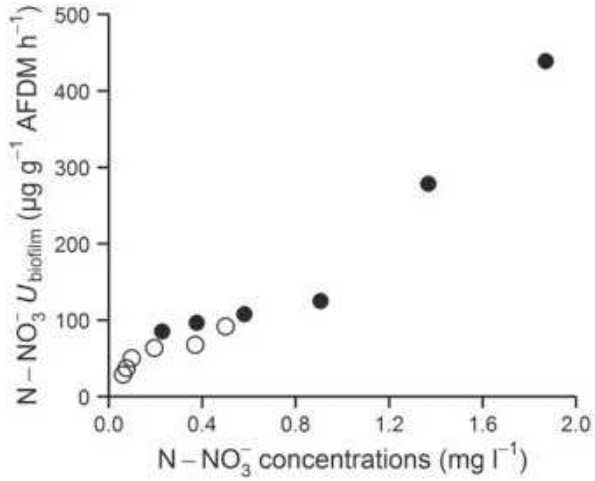


Fig. 2-6 Relationship between concentration and $U_{biofilm}$ of N-NO_3^- in both NAT-BIOF and NUT-BIOF together. Hollow points: NAT-BIOF, solid points: NUT-BIOF

2.6 Discussion

2.6.1 Effects of nutrient enrichment on biofilm-dwelling meiofauna

The effects of nutrient enrichment on meiofauna are well documented in both freshwater and marine sediments, but rarely in biofilms. Recently Kazemi-Dinan et al. (2014) compared the biofilm-dwelling meiofauna community across different lake trophic states and highlighted that nematode density and functional richness correlate positively with nutrient availability. This paper provided a rationale that changes in nutrient loads primarily affect the composition of microbial communities and that bottom-up effect is differently transmitted to meiofaunal taxa, based on their feeding preferences. The results of the studies in sediments are somewhat divergent. For instance, Wormald & Stirling (1979) observed an increase in

density of marine sediment-dwelling nematodes with phosphate or nitrate enrichment after 38 to 70 days. Recently, Mitwally & Fleeger (2013) reported inconsistent and variable responses of densities of saltmarsh meiofauna to long-term (5 years) nutrient enrichment in marine muddy sediments. Ristau et al. (2013) observed that density of freshwater lake bacterial and algal-feeding nematodes increased in the nutrient-poor treatments in a 16-month long sediment-microcosm experiment. These recent findings suggest that meiofaunal responses are rather slow and can take months to years to develop (Hillebrand et al., 2002; Posey et al., 2002; Mitwally & Fleeger, 2013), and that long-term impacts of nutrient enrichment on density and/or biomass of meiobenthic invertebrates are context-dependant and comparatively weaker than the responses of meiofauna to other factors, such as temperature and biotic constraints (e.g. resource availability) (Hulings & Gray, 1976; Majdi et al., 2011; Ristau et al., 2013).

Studies of lacustrine meiofauna have shown that the density of lacustrine rotifers can increase with the increasing phosphorus concentrations in lake habitats (Särkkä, 1992; Ristau & Traunspurger, 2011; Wu et al., 2014) and in microcosm sediments (Ristau et al., 2012). Ristau et al. (2012) propose that the observed responses are indirectly linked to a nutrient-induced change in the availability of food (e.g. of unicellular diatoms and green algae) in experimental treatments. By comparison with our microcosms where bacterial density increased with nitrate enrichment but algal biomass did not, our results suggest that rotifer density and biomass indirectly responded to nitrate enrichment through consumption of bacteria. Indeed, benthic rotifers can consume a wide variety of preys as algae, bacteria and yeast (e.g. Ricci & Balsamo, 2000; Duggan, 2001; Mialet et al., 2013). Moreover, previous studies report that the response of lacustrine meiofauna to nutrient addition differs among meiobenthic taxa (Särkkä, 1992; Wu et al., 2004; Ristau & Traunspurger, 2011; Ristau et al., 2012). Our results support these findings. Although in the Garonne River, meiofauna consist mainly of nematodes from the family Chromadoridae (*Chromadorina bioculata* and *Chromadorina viridis*) and bdelloidae rotifers (Majdi et al., 2012a), only rotifer density was increased by nutrient enrichment within the 5 days of our experiment. This was likely due to lower rates of population turnover of nematodes compared to rotifers which have parthenogenetic reproduction and short time life cycles allowing them to show quick community responses to improving ambient conditions (Ricci & Balsamo, 2000; Majdi et al., 2012a). Considering a larger time-window would have been more appropriate to detect responses of nematode populations to nutrient enrichment.

Nevertheless, our study shows that in rivers, where rotifers are important contributors to the biofilm-dwelling meiofauna (Reiss & Schmid Araya, 2008; Kathol et al., 2011; Majdi et al., 2012a), biofilm lotic meiofauna can potentially react rapidly to short-term nutrient enrichment (e.g. short-term nutrient pulses after rainfall-induced runoff from agricultural catchments). We suggest that bacterial biomass increase enhanced food availability for biofilm-associated rotifers. The short-term response of meiofauna to nutrient enrichment has been previously overlooked and our results provide a first assessment of this response.

2.6.2 Nitrate uptake and kinetics

Our results support previous field enrichments experiments reporting that, in streams, nutrient uptake increases as environmental nutrient concentrations are increased (e.g. Dodds et al., 2002; Earl et al., 2006). Concerning uptake kinetics, when enrichment experiments use a given community, results often suggest that Michaelis-Menten model best fits DIN uptake kinetics (Payn et al., 2005; Earl et al., 2006; Covino et al., 2010; O'Brien & Dodds, 2010). Ribot et al. (2013) however, found that Michaelis-Menten model fit uptake of stream biofilms for NH_4^+ but not for NO_3^- in a channel experiment. Michaelis-Menten kinetics is characterized by saturation of uptake meaning that availability exceeds biological demand (Earl et al., 2006). In our results, biofilm uptake rate of nitrogen seemed to reach a plateau under low nutrient concentrations but it tended to increase under higher $N\text{-}NO_3^-$ concentrations. Such differences in biofilm response (i.e. with or without saturation kinetics) have been previously reported. O'Brien & Dodds (2010) proposed that they were related to variations in biofilm biomass among the different streams considered in their study. However, this cannot explain our results since 1) we standardised $N\text{-}NO_3^-$ uptake rates for biofilm biomass and 2) we did not observe significant difference in biofilm biomass between treatments.

The lack of response of microalgal biomass to $N\text{-}NO_3^-$ enrichment could be due to the relatively high nitrate concentrations — ranging from 266 µg l^{-1} to 8857 µg l^{-1} (i.e. from 60 µg l^{-1} to 2000 µg l^{-1} of $N\text{-}NO_3^-$) — which were above the growth-limiting level for freshwater benthic algae i.e. > 50 to 100 µg l^{-1} (e.g. Stevenson et al., 1996) in both enriched and non-unenriched treatments**.** Alternatively, considering that rotifers are effective grazers in river biofilms (Kathol et al., 2011; Mialet et al., 2013) and algal raw production may increase without showing any changes in their biomass or density when rapidly ingested by rotifers, grazing of algae by increasing rotifer density might on the one hand favor bacteria in the competition for $N\text{-}NO_3^-$ and on the other hand keep the algal population in an active

growth phase and hence stimulate $N\text{-}NO_3^-$ uptake of the biofilms at high $N\text{-}NO_3^-$ concentration. We can hence not exclude that stimulated microalgal growth also participated to the increased $N\text{-}NO_3^-$ uptake in the enriched conditions.

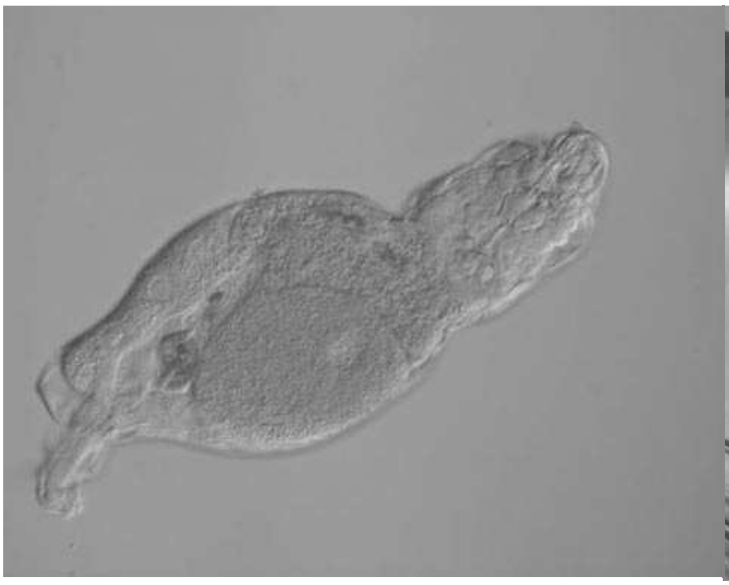
Conversely, bacteria responded to nitrate enrichment by a density increase. Nitrate uptake results from assimilatory processes (i.e. incorporating nitrate into biofilm biomass). Furthermore, in streams, apparent nitrate uptake may include dissimilatory transformations (in which N is not incorporated into biomass) such as denitrification (i.e. the respiratory process by which bacteria reduce NO_3^- to N_2) (Bernot & Dodds, 2005; Ribot et al., 2013). Considering the observed increase in bacterial density between NUT-BIOF and NAT-BIOF microcosms in our experiment, it is possible that denitrification also contributed to $N\text{-}NO_3^-$ decrease in the water (Lyautey et al., 2003). The observed uptake kinetics may result from a saturation of photosynthetic incorporation of nitrate in biofilm biomass which was likely relayed by an increase of heterotrophic bacterial transformations of NO_3^- (e.g. denitrification). This statement is supported by an experiment showing that uptake of nutrients in absence of leaf litter was longer relative to systems with a natural abundance litterfall (Webster et al., 2000)(Webster et al., 2000) supporting the idea that short-term retention of dissolved N is increased by heterotrophic organisms associated with organic matter (Bernot & Dodds, 2005). Proia et al. (2012) also observed significant response of bacterial density but not of algal biomass in microcosm biofilms. Despite this lack of algal growth, their results suggest that microalgal-bacterial interactions were enhanced by nutrient enrichment, as suggested by our experiments.

The most remarkable result of our experiment was the important and rapid increase in rotifer density and biomass in the enriched microcosms. This is most likely a result of nutrient-stimulated resources for the rotifers (probably both algae and bacteria). On the other hand, rotifers might also themselves have contributed to the stimulation of bacterial growth, by their bioturbation activity which could favour oxygen turnover and solute exchanges, as it has been shown for nematodes (Traunspurger et al., 1997; Riemann & Helmke, 2002; Teissier et al., 2007; Nascimento & Naslund, 2012). This is supported by the concomitant increase of bacterial and meiofaunal densities. The stimulated development of meiofauna through nutrient enrichment could, through a feedback, enhance the microbial communities and hence nutrient uptake rates of biofilms. This requires further investigations to be confirmed. Previous studies of interactions between invertebrates and biofilms suggest that macrobenthos grazing indirectly reduces the relative nutrient uptake efficiency of biofilms, by simplifying the composition of the biofilm community and by decreasing its biomass

(Sabater et al., 2002). Conversely, our results suggest that interactions between biofilm associated meiofauna and microbial community could indirectly favor the performance of biofilms in the amelioration of the quality of river water. The effect of nutrient enrichment on both meiofauna and microbial communities has been relatively well studied, particularly in lentic ecosystems (Särkkä, 1992; Wu et al., 2004; Ristau & Traunspurger, 2011; Ristau et al., 2012). Nevertheless, to the best of our knowledge, in phototrophic biofilms, the present study is the first to provide results suggesting a possible link between bacteria-meiofauna interactions and short-term N uptake capacity.

Chapter 3: Relation entre la densité des rotifères et le taux de consommation de $N\text{-}NO_3$ d'un biofilm épilithique de rivière

Article soumis dans *Freshwater Biology*, en révision



3.1 Résumé de l'article

3.1.1 Contexte et objectifs

Un enrichissement en nutriments peut induire une réponse à court terme des rotifères et des bactéries et une augmentation de la capacité du biofilm à consommer le $N\text{-}NO_3$ (Chapitre 2). Cependant, la question de savoir si la méiofaune peut influencer les processus du biofilm en relation avec sa capacité à consommer les nutriments, n'a pas été clairement élucidée. A ce jour, peu d'études se sont intéressées au rôle potentiel de la méiofaune dans les services écosystémiques comme par exemple les effets possibles de ses interactions avec les micro-organismes sur les processus liés à la capacité de consommation des nutriments par les biofilms. De plus, la plupart de ces études ont concerné le milieu marin (Gaudes et al., 2006; Näslund et al., 2010; Ackermann et al., 2011; Nascimento & Naslund, 2012; Bonaglia et al., 2014; Stock et al., 2014) Stock et al. (2014) par exemple, ont rapporté que les diatomées peuvent intensifier l'effet positif indirect (interactions avec les bactéries) de la présence des copépodes méiobenthiques sur la dénitrification dans des sédiments marins côtiers. Il apparaît donc que les interactions entre la méiofaune, les bactéries et les microalgues peuvent avoir un impact significatif sur les flux d'azote dans les écosystèmes aquatiques. Etant donné que les micro-organismes constituent le principal compartiment impliqué dans les processus de rétention des nutriments par les biofilms (e.g. Sabater et al., 2002; Cardinale, 2011), il est envisageable que les interactions méiofaune-bactéries-microalgues puissent contribuer à augmenter la capacité d'auto-épuration des biofilms en milieu lotique. Le Chapitre 3 examine cette question. Dans cette optique, l'étude expérimentale décrite dans ce chapitre reprend le principe expérimental présenté dans le chapitre 2, modifié notamment par l'intégration de microcosmes dont les biofilms ont été soumis à différents niveaux de densités de méiofaune. Des microcosmes ont été élaborés pour la cuture de biofilms en conditions lotiques, au laboratoire.

3.1.2 Principaux résultats et discussion

Dans l'ensemble des traitements, les rotifères dominaient la méiofaune associée aux biofilms. Les densités de rotifères et de bactéries ont été significativement corrélées dans les microcosmes enrichis en $N\text{-}NO_3$ pendant la période expérimentale. Aucune variation de la biomasse ni de la composition (en termes de concentration en chlorophylle *a* et en pigments biomarqueurs) de la fraction photosynthétique des biofilms n'a été observée pendant la période expérimentale.

Le taux de consommation de $N\text{-}NO_3$ par les biofilms était significativement plus élevé dans les traitements soumis aux plus hautes densités de rotifères relativement à ceux contenant les plus basses densités, pendant les deux premiers jours. Ces résultats montrent que les rotifères ont contribué à l'augmentation à court-terme, de la consommation du $N\text{-}NO_3$ par les biofilms, dans les microcosmes enrichis en nutriments.

Considérant que les densités en rotifères et bactéries ont été significativement corrélées, ces résultats suggèrent que (1) les rotifères ont pu indirectement contribuer à la stimulation de la communauté bactérienne, et (2) que ces deux communautés ont interagi dans les microcosmes enrichis en nutriments, confortant ce qui a préalablement été suggéré dans le Chapitre 2 (Liu et al., 2015). Ces résultats concordent avec deux autres études récentes décrivant les effets significatifs à court terme, de densités élevées en méiofaune sur le cycle de l'azote dans des sédiments marins (Bonaglia et al., 2014; Stock et al., 2014). Bonaglia et al. (2014) par exemple, ont observé que des densités élevées en nématodes méiobenthiques produisent une augmentation de la production de diazote (par dénitrification), donc, potentiellement par interactions avec les bactéries dénitrifiantes de sédiments marins superficiels. Stock et al. (2014) indiquent que les interactions copépodes méiobenthiques - bactéries peuvent stimuler les processus d'incorporation des nitrates dans la biomasse microalgale de sédiments estuariens. Dans ce contexte, la présente étude suggère que l'augmentation remarquable du taux de consommation du $N\text{-}NO_3$, observée pour les biofilms des microcosmes aux densités de rotifères les plus élevées, résulterait de la stimulation indirecte de la croissance bactérienne par la méiofaune plutôt que d'un effet potentiel direct des rotifères. Les processus impliqués pourraient être d'une part, liés à l'activité de bioturbation de la méiofaune (Bonaglia et al., 2014) pouvant être potentiellement active dans les biofilms (Mathieu et al., 2007), et d'autre part, aux produits excrétés par les méio-invertébrés qui peuvent enrichir le milieu en substrats organiques, favorisant le développement des bactéries (Ferris et al., 1998; Moens et al., 2005). Ces deux processus, déjà étudiés pour les nématodes n'ont à ce jour, pas encore été mis en évidence pour les rotifères méiobenthiques.

Note: This manuscript has slightly been changed in comparison with the version submitted to the referees.

Presence of biofilm-dwelling rotifers increase the specific $N–NO_3^-$ uptake rate of a river biofilm

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3.2 Abstract

1. The ability of stream biofilms to improve water quality (*e.g.* excessive nitrogen loads) is well known, and microorganisms *e.g.* microphytobenthos and bacteria are involved in the biofilm self-depuration processes. Meiofauna can influence primary productivity and stimulate bacterial growth and its significant role in nitrogen cycling has been recently shown in marine sediments. We hypothesized that biofilm-dwelling meiofauna indirectly influence biofilm functions related to nutrient uptake.

2. Biofilms originated from natural river water were cultured in flume microcosms. The $N\text{-}NO_3^-$ uptake rates of the biofilms as well as the response of microorganisms were studied under different conditions of meiofaunal density and $N\text{-}NO_3^-$ concentration over a 10-day experiment.

3. Rotifers strongly dominated the meiofaunal community. During the first two days of the experiment, the $N\text{-}NO_3^-$ biofilm uptake rate was increased in the high meiofaunal density treatments compared with the low meiofaunal density ones. This shows that high density of rotifers contributed to enhance the nitrogen uptake capacity of phototrophic biofilms at short-term. Density of rotifers and bacteria were positively correlated suggesting that these two communities interacted over the experimental period.

4. Overall, our results show that high density of rotifers can contribute to enhance nitrogen uptake capacity of phototrophic biofilms and strongly suggest that this occurs through their interactions with bacteria. It is thus likely that the presence of meiofauna in biofilms can contribute to limit nitrogen loads in river waters. This study supports the

hypothesis that the potential interactions between faunal groups and microbial communities of biofilms merit further investigations to improve our understanding of processes which regulate interactions between biofilms and the overlaying water in rivers.

Keywords:

meiobenthic rotifers; biofilms; bacteria; nitrate retention; rivers; self-purifying capacity

3.3 Introduction

Rivers are important inland water ecosystems and provide freshwater to human as a "provisioning" ecosystem service (Aylward & Bandyopadhyay, 2005; Elsin et al., 2009). However, increasing loads of dissolved inorganic nitrogen (DIN) in riverine systems have been observed worldwide (McIsaac et al., 2001; Green et al., 2004), which could lead to ecosystem degradation and biodiversity loss (Thompson et al., 2012). Recently, increasing attention is being paid to the natural ability of ecosystems to reduce pollution and render services to humans, the so-called ecosystem services (Daily, 1997; Millenium Ecosystem Assessment, 2005). Point and diffuse DIN sources, such as human sewage, deposition of air pollutants and some agricultural practices could induce environmental changes resulting in harmful consequences on ecosystem structure and functioning (Vitousek et al., 1997; Smith et al., 1999; Simon et al., 2004; Camargo & Alonso, 2006). Therefore, ecological processes reducing N concentrations in aquatic systems are considered as ecosystem services.

River epilithic biofilms are a complex assemblage comprising microphytes, bacteria, meiofauna and macrofauna embedded in a mucous matrix of exopolymeric substance (EPS) together with entrapped allochthonous material (e.g. Lock et al., 1984; Costerton et al., 1995; Romaní et al., 2003). This biofilm grows on any hard submerged substrate and, when enough light is available, microphytobenthos (and their EPS exudates) contributes to the organic content of biofilm (Azim et al., 2005). The benthic biofilm biomass at the surface of the sediment can be largely extended in the hyporheic zone when it exists. This attached biofilm is recognized to be the main driver of the carbon and nutrients uptake required for biomass production and respiration and sustain secondary production (e.g. Lock et al., 1984; Pusch et al., 1998; Baker et al., 2000; Battin et al., 2003; Cardinale, 2011). They can act not only as a sink for nutrients in the sediment/water interface, but also as a buffer against increasing nutrient concentrations (*e.g.* nitrate). Thus, they play an important role in the river self-depuration processes (Pusch, 1996; Sabater et al., 2002; Battin et al., 2003; Teissier et al., 2007; Liu et al., 2015). Among the involved processes, short-term nitrate retention via assimilatory uptake by biofilms could be notable as nitrate is intensively recycled within benthic communities (e.g. Burns, 1998; Bernot & Dodds, 2005). Bacterial denitrification (*i.e.* respiratory process reducing NO_3^- to N_2) in biofilms contribute to apparent uptake of nitrate in streams (Bernot & Dodds, 2005; Ribot et al., 2013). Among the environmental factors which drive the biofilm nitrate uptake processes, the ambient nitrate availability could be among the more important ones *i.e.* increase in nitrate availability could either enhance or

inhibit biofilm uptake (e.g. Schiller et al., 2007; Mohanakrishnan et al., 2009; Ribot et al., 2013; Liu et al., 2015).

Most studies dealing with the effects of fauna on benthic biogeochemistry *e.g.* the N cycle, have considered large animals because they are easy to manipulate in the laboratory and are expected to physically alter microbial pathways through bioturbation. Benthic macrofauna (invertebrates size > 1 mm) is widely recognized to play an important role in the regulation of carbon mineralization, nutrient regeneration and coupled nitrification/denitrification (Aller, 1994; Lillebø et al., 1999; Gerino et al., 2003; Gilbert et al., 2003; Welsh, 2003). Macrofaunal activity is known to either enhance denitrification due to particle reworking and burrowing, ventilation and bioirrigation (Karlson, 2007; Stief, 2013), or have negative impact on denitrification by means of dissimilatory nitrate reduction to ammonium (Bonaglia et al., 2013). It is suggested that benthic macrofauna can negatively affect biofilm processes related to their nutrient uptake ability due to consumption and reduction of biofilm biomass (e.g. Sabater et al., 2002; Barranguet et al., 2005). In contrast, few papers have dealt with the impact of meiofauna i.e. benthic invertebrates with a body size ≤ 500 μm (Giere, 2009) on benthic ecosystem processes *e.g.* its effect on sediment biogeochemistry and its interactive effects with other microorganisms on biofilm depuration functions. Published studies have largely focused on marine systems (Gaudes et al., 2006; Näslund et al., 2010; Ackermann et al., 2011; Nascimento & Naslund, 2012; Bonaglia et al., 2014; Stock et al., 2014). Meiofauna are extremely abundant in epilithic river biofilms (Gaudes et al., 2006; Kathol et al., 2011; Majdi et al., 2011; Liu et al., 2015). Despite of their low grazing pressure on biofilm microphytobenthos (Majdi et al., 2012a; 2012c; Mialet et al., 2013), meiofaunal activity within the biofilm matrix could affect oxygen cycling and enhance primary productivity through their meio-bioturbation activity (Mathieu et al., 2007). Liu *et al*. (2015) suggest that meiofauna could stimulate the growth of bacteria in epilithic biofilms. This supports previous findings (Riemann & Helmke, 2002; Nascimento & Naslund, 2012; Stock et al., 2014) which have shown that marine nematodes influence nitrogen cycling in sediments only when they interact with bacteria. Stock *et al*. (2014) discovered that diatoms also enhanced the effect of copepods on denitrification in sediments, thus showing that interactions between bacteria, meiofauna and algae have an important impact on marine sediment nitrogen fluxes. Since microorganisms *e.g.* microphytobenthos and bacteria, are the main organisms directly involved in nutrient retention processes in biofilms (*e.g.* Sabater *et al.* 2002; Cardinale 2011), it can be expected that the positive interactions between meiofauna

and microorganisms improve the self-depuration functions of biofilm in natural running waters.

Stream biofilms can adapt their nitrate uptake rates according to nitrate availability and speciation in the environment (Bernot & Dodds, 2005; Ribot et al., 2013). Relatively higher nitrate availability can induce increases in the density of marine and freshwater sediment/biofilm-dwelling meiofauna (Mitwally & Fleeger, 2013; Ristau et al., 2013; Liu et al., 2015). Since it has been shown that meiofauna can influence primary productivity and stimulate bacterial growth, we hypothesized that biofilm-dwelling meiofauna could indirectly influence biofilm functions related to nutrient uptake. We have previously shown that rotifers can rapidly respond to nitrate enrichment and suggested a possible link between bacteria-meiofauna interactions and the short-term N uptake capacity of biofilms (Liu *et al.* 2015). As a second step, this study aims to test the hypothesis that increased meiofauna density stimulates nitrate uptake processes of stream epilithic biofilms.

3.4 Methods

3.4.1 Experimental design

Microcosm – The experiments were carried out in a microcosms designed to mimic a river scenario. The microcosms consisted of flumes made of Plexiglas, PVC tubes and submerged pumps (flume size: length 80 cm, width 6 cm, height 8 cm). As shown in Fig. 1, a micro-reservoir (length 8 cm, width 8 cm, depth 4 cm) set up at the upstream end of the flume was used to smooth water flow, and a bottom-reservoir (length 10 cm, width 10 cm, depth 10 cm) was used to store water and pump it up to the micro-reservoir. The flow rate was controlled to 2.5 L min^{-1}. The average volume of water circulating in each microcosm was 2.61 ± 0.03 L.

Treatment design – As shown in Fig. 1, our experiment had five types of flume treatments: (1) non-enriched Garonne water (further called river water) without biofilm (NAT), (2) without biofilm incubated with nutrient-enriched water (NUT), (3) non-enriched river water in which a biofilm was allowed to develop (NAT-BIOF), (4) nutrient-enriched river water in which a biofilm was allowed to develop (NUT-BIOF), (5) nutrient-enriched river water, with biofilm and enriched in meiofauna (NUT-BIOF+). All treatments had four replicates. After the period of biofilm growth, all microcosms were incubated for 10 days under controlled conditions (see below).

Biofilm growth – Water and epilithic biofilm were sampled from the Garonne River, 30 km upstream of Toulouse (location: 01°17' 50" E, 43°23'43" N; elevation: 175 m asl). For biofilm sampling methods see details in Majdi *et al*. (2012a) and briefly stated here: (1) sampling of cobbles into a plastic bag underwater (depth = 30-50 cm); (2) transporting the cobbles to the laboratory in a cool box within 2 h; (3) removing the epilithic biofilm by scraping with a scalpel and a toothbrush. These samples were used to obtain a 5 L biofilm suspension in culture medium for the growth of microalgae following Dauta (1982). Meanwhile, river water was filtered with 50 µm mesh to remove zooplankton and suspended meio- and macrofauna, but not the bacteria and microalgae. Filtered water was added into the microcosms. Biofilms were cultured under experimentally controlled conditions *i.e.* 17 °C; light:dark 12:12 h, 105 µmol m^{-1} s^{-1} neon lights. The concentrated biofilm suspension was diluted with the filtered river water and homogenized. A 1 L subsample of this diluted biofilm suspension was poured into each of the microcosms to allow early biofilm colonizers to settle on the flume substrate (Plexiglas). The biofilm growth period lasted for one and half month before the start of the experiment.

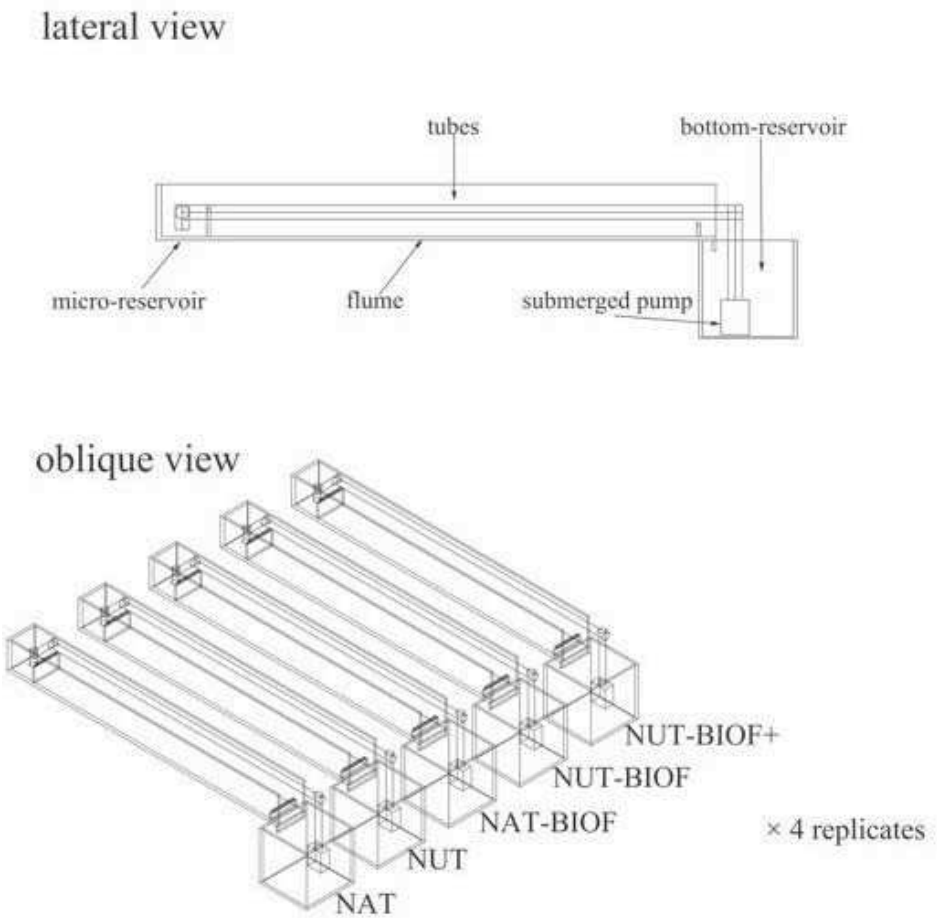

Fig. 3-1 The microcosms and experimental design

Nitrate enrichment – The $N\text{-}NO_3^-$ concentration in the Garonne River generally ranges from 0.6 to 24 mg L^{-1} (Ameziane et al., 2002; Leflaive et al., 2008). After the period of biofilm growth, the water in the microcosms was carefully replaced by new filtered river water by pumping water out of the bottom-reservoir and adding new water to it. We used 50-µm-mesh filtered river water for non nutrient-enriched treatment (*i.e.* NAT and NAT-BIOF). At the beginning of the experiment *i.e.* 2 h before the day 0 sampling (see below), nitrate was added to the river water in nutrient-enriched treatments, to mimic downstream eutrophic condition as indicated in a previous study reporting DIN concentrations > 2 mg L^{-1} (Muylaert et al., 2009). $N\text{-}NO_3^-$ final concentrations in microcosm water at the beginning of the experiment were as following: in NAT and NAT-BIOF, 0.59 ± 0.18 mg L^{-1} (n = 8, ± SE); in NUT, NUT-BIOF and NUT-BIOF+, 2.70 ± 0.11 mg L^{-1} (n = 12, ± SE).

Meiofauna enrichment – Living meiofauna was extracted from sediment samples collected in the Garonne River following the procedure described by (Giere, 2009; Nascimento & Naslund, 2012) with some minor modifications. The top 5 cm sediment was collected by carefully scraping using a trowel and sieved on 1 mm and 50 µm meshes. The meiofauna retained on the 50 µm sieve were anesthetized by 5 min immersing in an isotonic solution of 0.75 mol L^{-1} $MgCl_2$. Then, meiofauna samples were rinsed (on 50 µm mesh) with filtered river water and mixed with 500 mL Levasil 40% colloidal silica solution. This allowed to separate organic matter including living meiofauna from sediment particles by air bubbling for 1 min to facilitate floatation of meiofauna. After settling for 5 min, the supernatant containing the meiofauna was rinsed thoroughly with filtered river water. The procedure was repeated twice for each batch of sediment. The meiofauna suspension was gently homogenized and divided in four equivalent volumes which were added in the upstream of the four NUT-BIOF+ flumes. Before starting the experiment, samples from each flume with biofilm were collected for meiofauna counting, to ensure the significantly higher meiofauna density in NUT-BIOF+ flumes. The gap between the day when meiofauna was added and the day 0 of the experiment was 7 days.

Sampling scheme – The experiment started on June 2nd 2014 *i.e.* day 0. For all treatments, day 0 sampling was carried out after a 2 h period of stabilization following $N\text{-}NO_3^-$ addition in NUT-treatment microcosms. Both water and biofilm samples were collected in microcosms on 0 d, 2 d, 5 d and 10 d. Only water samples were taken in the control microcosms (*i.e.* NAT and NUT). One mL of water was sampled from each microcosm and filtered (0.22 µm PTFE syringe filter) for nutrient analyses. Biofilm was sampled using a 5 mL plastic tube which was applied to the bottom (sampled area: 7.54 cm^2). A small scraper

was used to scratch biofilm off the microcosm bottom within the tube area. During the scratching, the tube remained being pressed carefully to the bottom to minimize external water intrusion. One sample was taken for each microcosm and for each sampling occasion. Samples were taken from the downstream end to the upstream end of each microcosm. Then, the biofilm sample was sucked using a 5 mL syringe and mixed (1:5) with filtered river water (30 mL final volume). These 30 mL biofilm suspension samples were preserved in formaldehyde solution (5 % final concentration) for measurements of bacterial and meiofaunal densities or stored at -80°C for pigment analyses (see below). All samples were in 4 replicates. Deionized water was added to compensate the water evaporation in the microcosms every 3 days.

3.5 Sample treatments

Chloride (Cl^-) concentration was measured to evaluate evaporation in microcosms. Cl^- and N concentrations ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$) were analyzed in water samples by high-performance ionic chromatography (Dionex DX-120, Thermo Fisher Scientific Inc., Waltham, MA, USA) following standard procedures (NF EN ISO 10304-1, 1995). For meiofaunal density counting, two drops of 1 % Rose Bengal stain were added into 5 mL biofilm suspension subsamples. Meiofauna was counted in a Dolfuss cell (Elvetec Services, Clermont-Ferrand, France) under a stereo microscope (9-90×).

For bacterial density analysis, each biofilm suspension preserved in formaldehyde solution was homogenized and a 20 µL subsample was used for a standard DAPI-staining analysis (Porter & Feig, 1980). Each subsample was gently sonicated to maximize detachment of bacterial aggregates prior to staining and counting (Buesing & Gessner, 2002). Bacterial counting was carried out under a Leitz Dialux microscope (1,250×) fitted for epifluorescence: HBO 100 W mercury light source (Osram, Winterthur, Switzerland), with an excitation filter for 270 and 450 nm, a barrier filter of 410 and a 515 nm cut-off filter.

For the HPLC measurement of biomarker pigments in the biofilm algal community, each 5 mL subsample of biofilm suspension was freeze-dried to remove excess water (Buffan-Dubau & Carman, 2000) and sonicated (15 min in a cold bath) in a total of 5 mL 98 % cold-buffered methanol with 2 % of 1 M ammonium acetate with ultra-sonication bath (Sonifier 250A, Branson Ultrasonics corp., Danbury, CT, USA) to extract pigments. The extraction was repeated once and both pigment extracts (*i.e.* 10 mL total volume) were pooled, filtered on 0.2 µm PTFE syringe filter and analyzed using a high performance liquid chromatograph (HPLC) consisting of a 100 µL loop auto-sampler and a quaternary solvent

delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies inc., Santa Clara, CA, USA). The mobile phase was prepared and programmed according to the analytical gradient protocol described in Barlow *et al*. (1997). Pigment separation was carried out through a C18,5 µm column (MOS-2 HYPERSIL, Thermo Fisher Scientific Inc.). ChemStation software (version A.10.02, Agilent Technologies Inc.) was used for data analyses. Pigments were identified by comparing their retention times and absorption spectrum with those of authentic standards (DHI LAB products, Hørsholm, Denmark). For pigment quantification, a standard response factor was calculated from the linear relationship between the concentration and the corresponding peak area on HPLC chromatograms.

For the estimation of the relative contribution of different microalgal groups *i.e.* diatoms, green algae and cyanobacteria to total microphytobenthic biomass in terms of chlorophyll.*a* (Chl.*a*), CHEMTAX version 1.95 software (Mackey et al., 1996) was used to perform chemotaxonomic analysis. The values of the biomarker pigment ratio to Chl.*a* for each microalgal group of the Garonne biofilm and the detail of the procedure are provided by Majdi *et al*. (2011).

For the quantification of biofilm biomass, each 5 mL biofilm suspension subsample was dried overnight at 55 °C, weighted to obtain the dry weight (DW) and further combusted during 8 h at 450 °C to obtain the ash weight (AW). Ash-free dry mass (AFDM) was calculated as DW – AW.

3.6 Data analysis and statistics

The procedure of calculation for nitrate biofilm uptake rate was modified from Liu *et al*. (2015) as described below. Variations in chloride (Cl^-) concentrations between two subsequent sampling occasions were used to correct the $N\text{-}NO_3^-$ concentrations for potential increase caused by both water evaporation and water loss by the previous samplings as Eq. 1:

$$C_t' = C_0' \times (C_{t(Cl)} / C_{0(Cl)})$$

where C_0' and C_t' (mg L^{-1}) are the $N\text{-}NO_3^-$ concentrations before and after correction at a given time (t in hours), respectively; $C_{0(Cl)}$ is the initial Cl^- concentration; $C_{t(Cl)}$ is the Cl^- concentration at a given time (t in hours).

We calculated the $N\text{-}NO_3^-$ uptake rates measured in the water phase of the microcosms as U (µg h^{-1}) with Eq. 2:

$$U = V \times (\Delta C_{\Delta t} / \Delta t)$$

where V is the volume of water in each microcosm in liter, Δt is time difference (in hours) between two sampling days. $\Delta C_{\Delta t}$ is $N\text{-}NO_3^-$ concentration difference (in mg L^{-1}) between mean concentrations in the water of the microcosms at two sampling days. $\Delta C_{\Delta t}$ was converted to µg L^{-1} for calculation of $N\text{-}NO_3^-$ uptake rates. The $N\text{-}NO_3^-$ uptake rates calculated for the water of the microcosms with/without biofilm were named U_{BIOF} as ($U_{NAT\text{-}BIOF}$, $U_{NUT\text{-}BIOF}$, $U_{NUT\text{-}BIOF+}$) and U_{WAT} as (U_{NAT}, U_{NUT}), respectively.

We calculated the $N\text{-}NO_3^-$ specific uptake rates realized by the biofilm as $U_{biofilm}$ (µg g^{-1}AFDM h^{-1}) with Eq. 3:

$$U_{biofilm} = (U_{BIOF} - U_{WAT}) / AFDM_{biofilm}$$

where $AFDM_{biofilm}$ is the mean ash-free dry mass of the biofilm (g cm^{-2}) at each sampling time in the microcosms (*i.e.* NAT-BIOF, NUT-BIOF and NUT-BIOF+ treatments). The total AFDM in these microcosms was calculated from the ratio between the sampling area (7.54 cm^2) and the entire microcosm area (408 cm^2).

Differences in variables between treatments were analyzed by one-tailed *t* test. Corrections (*e.g.* Bonferroni) were not undertaken here, in accordance with the suggestions regarding their suitability for ecological data in which the statistical signal in the data is often subtle and thus potentially obscured by overconservative corrections (e.g. Cabin & Mitchell, 2000; Moran, 2003; Nakagawa, 2004). Assumption of homoscedasticity was tested with Levene's test. Data failing to fulfill homoscedasticity were log-transformed. One-way repeated-measure ANOVA was used to examine treatment effect on the variables. Pearson's test was applied to test correlations (1) between rotifer and bacterial densities, and (2) between time and the variables. Mann-Kendall test was used to check the stability of $N\text{-}NO_3^-$ concentration in controls. Between replicates, variability was quantified as standard error.

3.7 Results

3.7.1 Nitrogen concentration

As expected, mean $N\text{-}NO_3^-$ concentration in controls was higher in NUT than in NAT treatments (3.43 ± 0.19 mg L^{-1} against 0.95 ± 0.13 mg L^{-1} respectively $P < 0.001$) and did not vary significantly throughout the 10-days experiment (Fig. 2a) ($P > 0.05$ Mann-Kendall test). At day 0, mean $N\text{-}NO_3^-$ concentrations in NUT-BIOF and NUT-BIOF+ were significantly higher than in NAT-BIOF treatments ($P < 0.001$, Fig. 3-2a). $N\text{-}NO_3^-$ enrichment was thus significant in NUT treatments at the beginning of the experiment. However, these

concentrations declined significantly between 0 and 2 d, and were not significantly different between NAT-BIOF, NUT-BIOF and NUT-BIOF+ at 2 d and 5 d. This showed that most of the $N\text{-}NO_3^-$ was consumed within the first two days. Thus, 0, 2 d and 5, 10 d can be characterized as periods with high and low nitrate concentrations respectively.

Table 3-1 P values from one-way repeated-measure ANOVA to examine "treatment" effect on each parameter. The letters a, b, ab, are from the results of multiple *t* test showing the significant differences between treatments

Parameters	ANOVA	Multiple *t* test		
	P value	NAT-BIOF	NUT-BIOF	NUT-BIOF+
Rotifer density	< 0.05	b	ab	a
AFDM	< 0.01	b	a	b
Bacteria density	> 0.05	a	a	a
Chlorophyll.*a*	> 0.05	a	a	a
Green algae %	> 0.05	a	a	a
Cyanobacteria %	> 0.05	a	a	a
Diatoms %	> 0.05	a	a	a

$N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ in NAT-BIOF, NUT-BIOF and NUT-BIOF+ treatments were mostly not detectable at all sampling occasions (missing values > 87.5 %). Those concentrations in controls (NAT and NUT) did not differ across time ($P > 0.05$, Mann-Kendall test), and no difference was found between NAT and NUT ($P > 0.05$). The mean $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ concentrations were 0.043 ± 0.0096 mg L^{-1} and 0.21 ± 0.13 mg L^{-1}, respectively.

3.7.2 Biofilm biomass

As shown in Fig. 3-3a, significant increases of the biofilm biomass (AFDM) with time were observed in NUT-BIOF ($P < 0.05$, $r = 0.50$) and NUT-BIOF+ ($P < 0.01$, $r = 0.65$) respectively, but not in NAT-BIOF ($P > 0.05$, $r = 0.26$). At 10 d, significantly higher biofilm biomass was found in NUT-BIOF than that in NAT-BIOF (Fig. 3-3a). This indicates that $N\text{-}NO_3^-$ enrichment had a positive effect on biofilm growth. There was a significant treatment effect *i.e.* NUT-BIOF+ < NUT-BIOF (Table. 3-1). This was due to the significantly higher biofilm biomass found in NUT-BIOF compared with NUT-BIOF+ at 0 and 5 d, as well as a slight higher tendency towards significance of that in NUT-BIOF at 2 d ($P > 0.05$, $t = 1.05$, Fig. 3-3a). The fact that meiofauna was added 7 days before the start of the experiment could explain this difference as a grazing effect of rotifers on biofilm biomass before 0 d.

3.7.3 Meiofaunal density

Rotifers dominated the meiofaunal community of biofilms in term of density: at 0 d, rotifers averaged 3.57 ± 1.65 10^3 ind. cm^{-2}, and, nematodes = 15.40 ± 8.35 ind. cm^{-2}, (*i.e.* 99.8 % and 0.2 % of the total density respectively). So, rotifers are the main focus of this paper. Significant increases of rotifer density with time were observed in NAT-BIOF ($P < 0.05$, $r = 0.51$) and NUT-BIOF ($P < 0.001$, $r = 0.82$) respectively, but not in NUT-BIOF+ ($P > 0.05$, $r = 0.031$). It suggests that the rotifer density in NUT-BIOF+ reached a plateau before 0 d. Rotifer density was significantly higher in NUT-BIOF+ than that in NUT-BIOF at 0 d ($P < 0.01$) indicating a successful meiofauna enrichment.

3.7.4 Bacterial density

Bacterial density in biofilms significantly increased with time in NAT-BIOF ($P < 0.001$, $r = 0.82$) and NUT-BIOF ($P < 0.001$, $r = 0.75$) respectively, but not in NUT-BIOF+ ($P > 0.05$, $r = 0.15$). However, there was no treatment effect on bacterial density (Table.1). As shown in Fig. 3-3c, mean bacterial density of NUT-BIOF+ was significantly higher than in NUT-BIOF at 2 d ($P < 0.01$). No differences were found in bacterial density between NAT-BIOF and NUT-BIOF ($P > 0.05$) at any of the sampling times, suggesting that $N\text{-}NO_3$ enrichment did not stimulate bacterial growth. Besides, bacteria and rotifer densities were strongly positively correlated in NUT-BIOF and NUT-BIOF+ (Fig. 3-4, $P < 0.001$, $r = 0.61$).

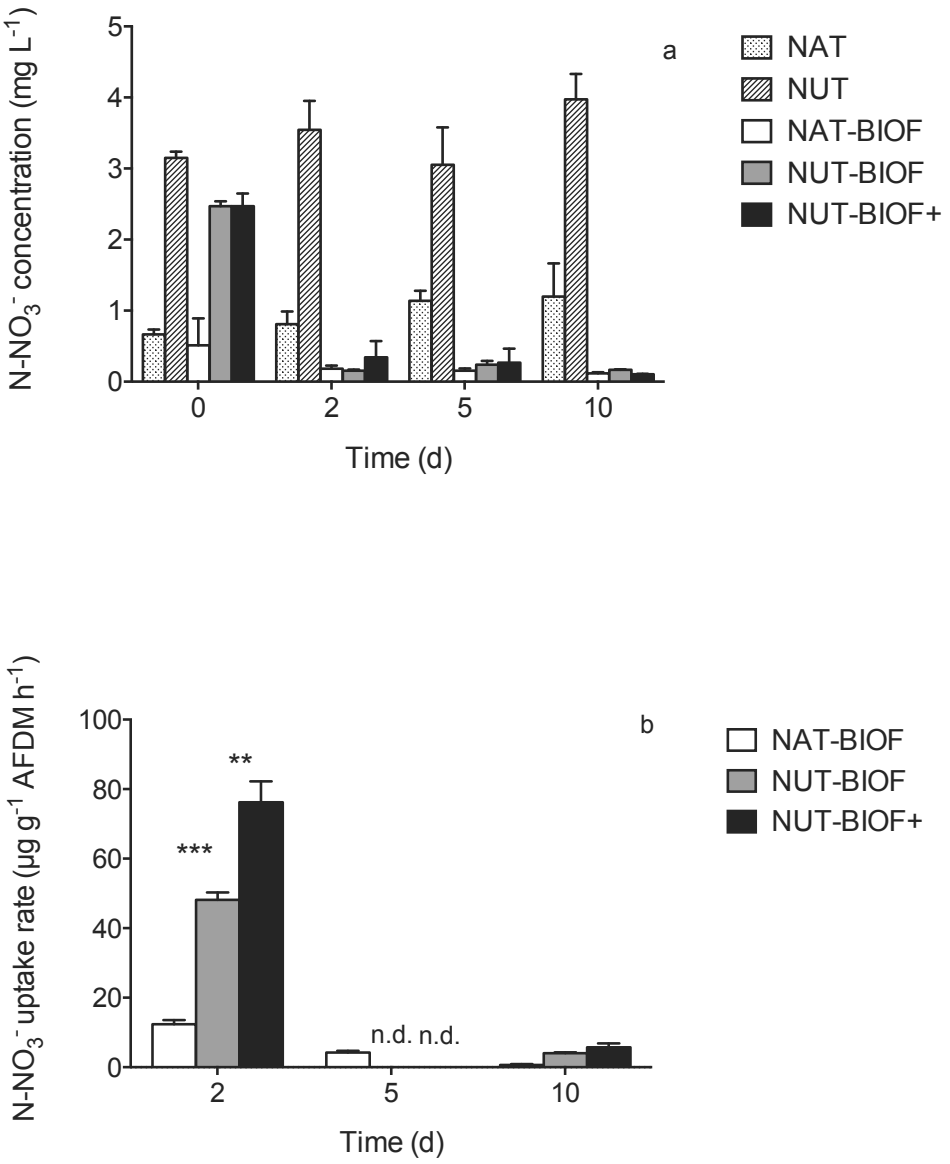

Fig. 3-2Averaged (a) $N\text{-}NO_3^-$ concentrations in water of each treatment, (b) $N\text{-}NO_3^-$ biofilm uptake rates in NAT-BIOF, NUT-BIOF and NUT-BIOF+ treatments (*i.e.* treatments with biofilm) at 0, 2, 5 and 10 d (*n* = 4 for each treatment at each time point). ** and * show significant difference between treatments at P < 0.01 and P < 0.001 respectively. "n.d." means there was no uptake detected in the treatment**

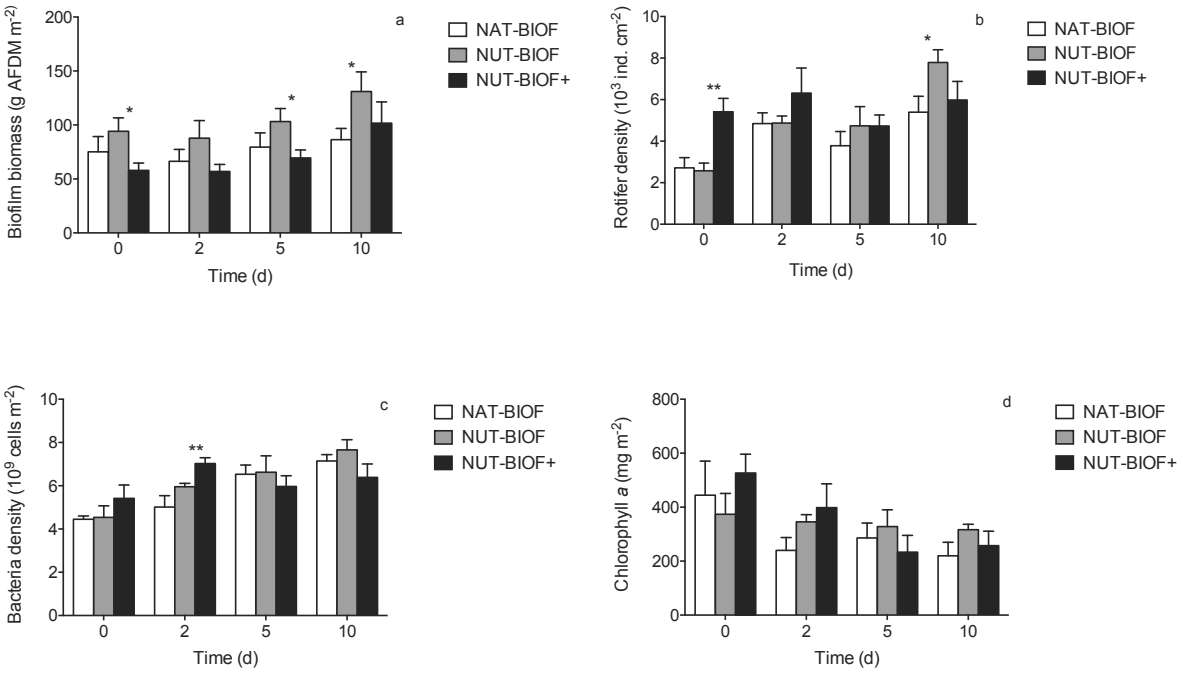

Fig. 3-3 Averaged (a) biofilm biomass, (b) rotifer density, (c) bacterial density, (d) chlorophyll.*a* concentrations in the biofilms of NAT-BIOF, NUT-BIOF and NUT-BIOF+ treatments at 0, 2, 5 and 10 d (*n* = 4 for each treatment at each time point). * and **show significant difference between treatments at P < 0.05 and P < 0.01 respectively

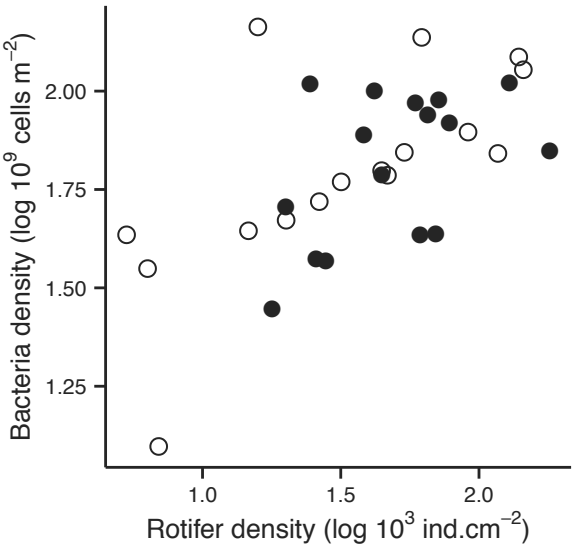

Fig. 3-4 Correlation between rotifer and bacterial density (samples from NUT-BIOF and NUT-BIOF+ at 0, 2, 5 and 10 d, *n* = 32, log-transformed data was used). White points NUT-BIOF, black points NUT-BIOF+

3.7.5 Pigment concentrations

Biofilm algal biomass, in term of chlorophyll *a* concentration, did not significantly differ among the treatments throughout the experimental period (Fig. 3-3d, Table. 3-1).

Among the 12 pigments which were present in biofilm samples, (1) fucoxanthin, (2) myxoxanthophyll and zeaxanthin, (3) lutein and chlorophyll *b*, showed that biofilm microphytobenthos comprised (1) diatoms, (2) cyanobacteria and (3) green microalgae. Green microalgae largely dominated the total algal biomass (on average 85 %) in all biofilm treatments while diatoms and cyanobacteria were similarly represented (on average 7 % and 8 % respectively. There was no statistically significant effect of time or treatment on algal concentration (Table. 3-1).

3.7.6 N-NO_3^- biofilm uptake rate

The N-NO_3^- biofilm uptake rates ($U_{biofilm}$) are shown in Fig. 3-2b. Averaged $U_{biofilm}$ was significantly higher in NUT-BIOF treatments than in NAT-BIOF at 2 d ($P < 0.001$) showing that difference in N-NO_3^- concentrations at 0 d induced a difference in uptake over the first 2 days. For N-NO_3^- enriched treatments, averaged $U_{biofilm}$ was significantly higher in treatments with high meiofaunal density (NUT-BIOF +) than in the treatments with low meiofaunal density at 2 d ($P < 0.01$). This shows that high rotifer density contributed to enhance $U_{biofilm}$ in nitrate enriched treatments for the first 2 days.

3.8 Discussion

Nutrient uptake by phototrophic biofilms generally increases with increasing dissolved inorganic nitrogen concentrations in rivers (Kim et al., 1990; 1992; Sabater et al., 2002; O'Brien & Dodds, 2010; Ribot et al., 2013; Liu et al., 2015). Our results support this finding and additionally, show that high density of rotifers in biofilms might also contribute to increase the nitrogen uptake capacity of phototrophic biofilms. Probably, at least a fraction of the nitrate taken up by biofilm organisms has previously been imported into the matrix by sorption. For instance, Freeman *et al*. (1995) mention that sorption of ionic nutrients to the biofilm matrix could increase nutrient retention time available for microbial metabolism. In the present experiment, we cannot distinguish the proportion of sorption mediated uptake from uptake by organisms directly from the water. The significant differences in uptake observed between "low" and "high" meiofauna treatments show that there are some biological processes involved, notably that the invertebrates influenced nitrogen uptake. Rotifer and bacterial densities were highly positively correlated in the biofilms suggesting (1) that rotifers may contribute to the stimulation of bacterial growth in biofilms, as previously stated by (Liu *et al.* 2015), and (2), that these two communities interacted. These results are in line with two recent short-term microcosm studies reporting significant effects of high meiofaunal density on nitrogen cycling in marine sediments. Bonaglia *et al*. (2014) observed that high density of marine nematodes indirectly enhanced the production of dinitrogen gas (during denitrification) in soft sediments. Stock *et al*. (2014) found that, in estuarine sediments, although benthic copepods (through interactions with diatoms) reduced denitrification rates, they also increased nitrate reduction to ammonium leading to assimilatory processes (*i.e.* incorporating nitrate into biofilm biomass), through interactions with bacteria. Both studies agree concluding that the observed effects were due to interactions between meiofauna and bacteria. In line with these studies, we suggest that the increase of nitrogen uptake observed for the high meiofauna density biofilms at 2 d was due, at least partly, to stimulation of bacteria, which influence nitrogen cycling rather than to a direct effect of rotifer activity. Such interactions between meiofauna and bacteria have been described for nematodes. They can result from meio-bioturbation in marine sediments (Bonaglia *et al.* 2014) and in biofilms (Mathieu et al., 2007) improving transport of solutes *e.g.* oxygen, ammonium and nitrate (Aller & Aller, 1992; Berg et al., 2001), and from products excreted by nematodes which may contain large amounts of nitrogen (Ferris et al., 1998; Moens et al., 2005) and favor bacterial development (Riemann & Helmke, 2002). Such

interactions have so far not been shown for meiobenthic rotifers. Nevertheless, it is known that benthic rotifers produce, for instance, a sticky substance used for temporary attachment (e.g. Ricci & Balsamo, 2000) which could be attractive for bacterial colonies as has been shown for nematodes (Riemann & Helmke 2002). Also, feeding activity of benthic rotifers on other bacterivorous organisms *e.g.* ciliates and heterotrophic nano-flagellates (Norf et al., 2009) might indirectly favor the growth of bacteria. Besides, Parent *et al.* (2001) showed that high populations of copepods resident in freshwater trickling filters with nitrifying bacterial biofilm could inhibit nitrification, probably because in these conditions, meiofauna can only feed on bacterial nitrifiers but not other food sources *e.g.* benthic algae (Parker et al., 1989; Andersson et al., 1994). Overall, it appears that the most abundant groups of meiofauna in ecosystems *i.e.* nematodes, rotifers and benthic copepods (Giere 2009) issued from different habitats and systems (*i.e.* river biofilm, estuarine sediments), can significantly influence nitrogen cycling.

In the present experiment, the period of nitrate uptake by biofilms was short (2 days), due to the rapid decrease in $N\text{-}NO_3^-$ concentrations in the microcosms with biofilm. So, this shows that meiofauna influenced positively nitrogen uptake on a short time-scale. Rapid colonization of a river biofilms regularly occurs in natural river biofilms. For example, after flood events which severely reduce or eliminate biofilm biomass and its associated meiofauna, natural biofilm growth covers relatively long periods during low-flow seasons, (*e.g.* 5 to 9 months in the Garonne river) where meiofaunal groups colonize the biofilm (Majdi et al., 2012b; Graba et al., 2014). This occurs according to different pathways; *i.e.* rotifers are efficient and rapid colonizers (Liu *et al.* 2015) faster than nematodes (Majdi *et al.* 2012b). The question remains whether the long-term occurrence of an abundant meiofauna community and the succession of rotifers and nematodes would also results in an increase in uptake rates of $N\text{-}NO_3^-$ by field biofilms. So far, this has not been tested and to the best of our best knowledge, the present study provides a first opening to this aspect. Additionally to different pathways of colonization, the behavior of meiofauna in the biofilm may vary among taxa; *e.g.* rotifers, using their pedal adhesive glands, have been observed dwelling and grazing on the upper layer of the biofilm (personal observations) whereas previous studies (Riemann & Helmke, 2002; Bonaglia et al., 2014) and microscopic observations (data not shown) suggest that nematodes mainly move inside the matrix modifying oxygen turnover in the biofilm (Mathieu *et al.* 2007). One can thus expect that these different behaviors among the two most abundant groups of biofilm-associated meiofauna (Ackermann et al., 2011;

Kathol et al., 2011; Majdi et al., 2012b), could influence uptake rates of $N\text{-}NO_3^-$ by the biofilm differently (at different extents). This would deserve further investigation.

Rotifer densities were relatively high in the microcosm biofilms (on average 4570 ± 1399 ind. cm^{-2} from all treatments) compared to the meiofauna abundance reported for biofilms in rivers as for example up to 500 ind. cm^{-2} in the river Llobregat, Spain (Gaudes et al., 2006); 877 ind. cm^{-2} in the river Rhine (Ackermann et al., 2011) and 487 ind. cm^{-2} in the river Garonne (Majdi *et al.* 2012b). The question whether meiofaunal activity may influence $N\text{-}NO_3^-$ uptake by biofilms at lower ranges of density arises from our results, however, two previous experiments provided indications supporting this hypothesis. Liu *et al*. (2015), using densities of rotifers averaging 20 ind. cm^{-2} have suggested a possible indirect effect of this meio-invertebrates on the short-term N uptake capacity of biofilms whereas Bonaglia *et al*. (2014) showed that nematodes indirectly enhanced denitrification in marine sediments at densities ranging between 68 and 71.8 ind. cm^{-2}. Considering that nitrate concentrations in river waters can be relatively stable during the low flow periods - *e.g.* 0.58 ± 0.1 mg L^{-1} on average from 06/07/2005 to 30/11/2005 in the Garonne River at our study site (Majdi *et al*, unpublished data) - it can be envisaged that during these periods when meiofauna and bacteria (*e.g.* denitrifiers) develop with biofilm biomass (Majdi et al., 2012a; Lyautey et al., 2013), meiofauna could efficiently facilitate the biofilm ability to remove nitrate in water through bacterial growth stimulation.

The lack of microalgal biomass and composition in responses to $N\text{-}NO_3^-$ enrichment was probably related to the relatively high $N\text{-}NO_3^-$ concentrations (averaging 3.43 ± 0.19 mg L^{-1} initially, and 0.13 ± 0.035 mg L^{-1} at 10 d) which were above the growth-limiting level for freshwater benthic algae *i.e.* > 50–100 µg L^{-1} (e.g. Grimm & Fisher, 1986; Lohman et al., 1991; Stevenson et al., 1996) as reported by Liu *et al.* (2015). This indicates that, $N\text{-}NO_3^-$ concentration was not a limiting factor for microalgal biomass at the beginning of the present study. Considering that lotic meiobenthic rotifers are effective grazers in river biofilms (Kathol et al., 2011; Mialet et al., 2013), the fact that microalgal biomass did not differ between treatments could indicate that rotifers grazed the primary production during the experimental period as suggested by Liu *et al*. (2015).

Teissier *et al.* (2007) have clearly identified a threshold of biofilm biomass value at 23 g AFDM m^{-2}, under initial DIN concentrations ranging from 0.4 to 2.3 mg N L^{-1}, for N-uptake processes happening in epilithic biofilm of the Garonne river. The authors shown that in thick biofilms (biomass > 23 g AFDM m^{-2}), denitrification dominates apparent NO_3 uptake (83 % of NO_3 removal) whereas in thin biofilms (biomass < 23 g AFDM m^{-2}), NO_3 uptake

mainly results from N algal uptake. Thus, the thin biofilms remove DIN from the water column at a higher extent than thick biofilms (2.5-fold higher per g of biomass) and so, have a storage function while thick biofilms can export N to the atmosphere as N_2. This supports that biofilms retain nutrients from the water column and are zones of transient storages (Sabater et al., 2002; Battin et al., 2003; Teissier et al., 2007). In the present study, initial concentrations of $N\text{-}NO_3^-$ (the main form of DIN in the microcosms) was 2.70 ± 0.11 mg L^{-1} in NUT-BIOF and NUT BIOF+, so, we could apply the threshold value provided by Teissier *et al.* (2007) to our study. It appears that both "low" and "high" meiofauna biofilms (NUT-BIOF and NUT-BIOF+) were thick (biomass > 23 g AFDM m^{-2}). This suggests that, in this study, the biofilm NO_3 uptake was mainly driven by heterotrophs (denitrification) rather than by phototrophs (algal uptake), and emphasizing the importance of the rotifer-bacteria interaction. This also suggests that the biofilm function of N export to the atmosphere as N_2 (through denitrification) was not limited by meiofauna at short-term. Besides, it is known that bacteria living in environments with nitrate limitation prefer nitrate ammonification over denitrification (Schmidt & Schaechter, 2012; van den Berg et al., 2015). In the present experiment, after 2 days, biofilm N uptake was strongly reduced, this could thus be due to the limited growth of denitrifiers when nitrate concentration is low. Moreover, to distinguish which group of bacteria associated with nitrate removal (e.g. ammonifiers *vs* denitrifiers) interacts more closely with high density of meiofauna deserves further study.

Benthic macrofauna has been described to negatively affect biofilm processes related to their nutrient uptake ability due to consumption and reduction of biofilm biomass (e.g. Sabater et al., 2002; Barranguet et al., 2005). The present study highlights that in contrast, meiofauna can positively affect biofilm $N\text{-}NO_3^-$ uptake through their interactions with bacteria and algae. Moreover, macrofauna can cause a decrease in both meiofauna activity and abundance in sediments due to disturbance, predation or competition for food (Alongi, 1985; Branch & Pringle, 1987; Olafsson, 2003), so, whereas macrofauna may indirectly facilitate the growth of bacteria and microalgae (Schmid Araya & Schmid, 2000; Simon et al., 2004). They can also limit the positive effect of meiofauna on biofilm $N\text{-}NO_3^-$ uptake. This is in line with conslusions of a recent study underlined that macrofauna (bivalves) counteracted the stimulating effect by meiofauna for the nitrifying and denitrifying microbial communities in marine sediments (Bonaglia et al., 2014). As a matter of fact, it strengthens the potential role of trophic cascade and competition interactions as regulator of N retention processes (Simon et al., 2004).

In conclusion, our results show that high density of rotifers can contribute to enhance nitrogen uptake capacity of phototrophic biofilms and suggest that meiofauna may contribute to limit nitrogen loads in rivers. This study supports that the potential interactions between faunal groups and microbial communities of biofilms merits further investigations to improve our understanding of processes which regulate interactions between biofilms and the overlaying water in rivers.

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Chapter 4: Rôle de la biodiversité dans les processus biogéochimiques à l'interface eau-sédiments de lits de rivière macroporeux : une approche expérimentale

Article submitted on *Ecological Engineering*

4.1 Resumé de l'article

4.1.1 Contexte et objectifs

Le milieu hyporhéique est une zone de transition entre les eaux souterraines et les eaux de surface des cours d'eau (Orghidan, 1959). Les sédiments hyporhéiques sont colonisés par un biofilm hétérotrophe (Battin, 2000) et des invertébrés, dont une méiofaune abondante (Schmid Araya, 2000). Ils sont le siège d'une activité biologique intense primordiale dans le fonctionnement des écosystèmes lotiques (Robertson & Wood, 2010). D'après le Chapitre 3, la méiofaune associée aux biofilms phototrophes peut contribuer à augmenter la capacité de consommation de $N\text{-}NO_3$ par ces biofilms, probablement par l'intermédiaire d'interactions avec les bactéries. Donc, il peut être supposé que de telles interactions avec leurs conséquences potentielles sur la consommation des nitrates par le biofilm, puissent se produire dans le milieu hyporhéique. En effet, il est suggéré que globalement, les interactions entre l'activité et la diversité des invertébrés et les fonctions microbiennes devraient être considérées comme des facteurs contribuant potentiellement à réguler les processus biogéochimiques (Nogaro et al., 2008), et donc notamment la capacité « d'auto-épuration » de l'hyporhéon (Nogaro et al., 2007). Dans ce contexte, la présente étude vise à tester l'effet potentiel de la biodiversité multicommunautaire (biofilm-méiofaune-macrobenthos) sur les taux de consommation des nitrates et du carbone organique dissous (COD) dans la zone hyporhéique. Le design expérimental est basé sur l'utilisation de microcosms permettant l'étude de colonnes sédimentaires d'après Mermillod-Blondin et al. (2011; 2011). Les consommations de $N\text{-}NO_3$ et de COD ont été suivies sous différentes conditions de diversité : contrôle abiotique (AS) ; biofilm (SB), biofilm + méiofaune (SBM), biofilm + méiofaune+ macrofaune (SBMM), pendant une période de 7 jours.

4.1.2 Principaux résultats et discussion

Le taux moyen de consommation de $N\text{-}NO_3$ a significativement augmenté en fonction des différents niveaux de biodiversité. De plus, la consommation du COD est apparue significativement plus élevée dans les traitements contenant la méiofaune (SBM) comparés aux traitements SB. Les résultats indiquent donc que la méiofaune et en particulier les rotifères, peuvent aussi jouer un rôle positif sur ces processus dans le milieu hyporhéique.

Le taux moyen de consommation de $N\text{-}NO_3$ est apparu significativement plus élevé dans les traitements SBMM relativement aux SBM montrant que l'addition de macrofaune peut intensifier l'augmentation de ce processus induit par la méiofaune, contrastant avec les

conclusions d'études précédentes. Par exemple, Bonaglia et al. (2014) ont montré que la présence du bivalve *Macoma balthica* contrebalance l'effet positif de la méiofaune sur la communauté microbienne associée aux processus de nitrification et dénitrification de sédiments superficiels marins. La présente étude met donc en évidence l'aspect potentiellement positif des interactions méiofaune – macrofaune pour les processus de consommation des nitrates dans les sédiments. De plus, en accord avec les conclusions des Chapitres 2 et 3, cette étude suggère fortement que l'effet des invertébrés résulte des interactions invertébrés-bactéries.

Role of biodiversity in the biogeochemical processes at the water-sediment interface of macroporous river bed: an experimental approach

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4.2 Abstract

Biological factors and their interactions are major environmental drivers that determine variations of the uptake and self-purification capacities of a stream riverbed. In these hydro systems, bioremedation processes are mainly driven by heterotrophic biofilms that colonize aquatic sediments. Since infauna bioturbation acts to modify the physical structure as well as the biological and chemical properties of the sediments, invertebrate communities can interfere with nutrient and organic matter uptake by biofilm via different ways. Invertebrate activities and feeding behaviour such as gallery digging or feeding on components of the biofilm generate porosity modifications and favour nutrient contact with biofilm in the interstitial media. In the context of nutrient concentration increase in surface waters due to human impacts, this paper focuses on biodiversity effects on nutrient uptake by sediments. The hypotheses tested are that (1) transformation of nutrients and dissolved organic matter is influenced by the presence of invertebrates, (2) the uptake of nutrients and

dissolved organic matter is more effective when the diversity of the benthic community increases.

These hypotheses were tested using microcosms reproducing water-sediment of river bed interface colonized with different levels of invertebrate biodiversity i.e. abiotic sediment (AS); sediment and biofilm (SB); sediment, biofilm and meiofauna (SBM); sediment, biofilm, meiofauna and macrofauna community assemblage, which corresponds to the total benthic community of a river bed sediment (SBMM). Uptake rates of nitrates ($N\text{-}NO_3^-$) and dissolved organic carbon (DOC) by sediments in the microcosms were measured and considered as a function of the different levels of biodiversity. Nitrate uptake rates increased significantly with increasing biodiversity level. After 56 days of biofilm development, $N\text{-}NO_3^-$ uptake rates ranged from 3.76 ± 0.35 mg N d^{-1} kg^{-1}sediment Fresh Weight (sed FW) in SB condition to 8.92 ± 0.69 mg N d^{-1} kg^{-1}sed FW in the treatment with the maximum biodiversity (SBMM). Denitrification rates increased by a factor of 6 in presence of meiofauna and macrofauna compared to those measured in sediment without invertebrates. DOC uptake rates also varied significantly with biodiversity levels but in a lesser extent than nitrate uptake rates (41.89 ± 2.24 mg C d^{-1} kg^{-1}sed FW with biofilm alone to 51.00 ± 1.39 mg C d^{-1} kg^{-1}sed FW with the addition of meiofauna community). Respiration increased by 40 % in presence of meiofauna and macrofauna compared to those measured in sediment without invertebrates. This study highlights the effects of interaction between microbial, macro- and meiofauna on $N\text{-}NO_3^-$ and DOC uptake in macroporous stream sediment.

Keywords:

Biodiversity; invertebrates; nutrient uptake; hyporheic zone; water-sediment interface; river bed

4.3 Introduction

Self-purifying capacity of rivers, as an important ecosystem regulating service, is defined as their ability to eliminate a perturbation and specially a chemical change in the dynamic equilibrium of the system (Streeter & Phelps, 1958). In a context of markedly increased nitrogen and carbon loadings in most of the surface water worldwide (Craig et al., 2008; Noe & Hupp, 2008), the study of the river self-purifying capacity associated to nutrient uptake by sediments remains a relevant research domain. A focus on nitrogen and carbon uptake capacities of rivers leads to identification of river compartments including their physical, chemical and biological properties that actively participate to the nutrient transformation pathways.

In rivers characterised by low water depth, large proportions of runs and riffles, high granulometry (mainly composed of pebbles/gravels), high current velocities and low residence times, these hydromorphological characteristics tend to limit biological and microbiological activities in the free flowing water and these conditions favour autotrophic biofilm development (Battin, 2000; Ameziane et al., 2002; Sauvage et al., 2003). However, when a hyporheic zone exists, the biofilm biomass may be largely extended with heterotrophic metabolisms in the sediment. This interstitial and attached biomass is composed of bacteria, protozoans and detritus. Biofilm is regarded as an important organic matter storage site and absorption site for dissolved organic matter (DOM) owing to its large internal surface area (Koutny & Rulik, 2007). It is recognized to be the main driver of the carbon and nutrient uptake required for biomass production and respiration (Baker et al., 2000; Battin et al., 2008). Nitrogen and carbon uptake rates are now established both for autotrophic biofilm (Mulholland et al., 2004; Teissier et al., 2007; Majdi et al., 2012b; Ribot et al., 2013) and for heterotrophic biofilm in gravel bed sediments (Dahm et al., 1998; Peyrard et al., 2008). The hyporheic zone, a transition zone between groundwater and streams (Orghidan, 1959), is now known as a site of high biological heterotrophic activity that is critical for stream ecosystem functioning (Boulton et al., 1998; 2010). It is an important site for mineralization of organic matter from surface waters. The importance of the hyporheic participation to the global nutrient processing in a river depends, among other factors, on the intensity of ground water/surface water (GW/SW) exchanges linked to the porosity or the clogging of sediment. The permanent water flow through these transition zones explains why hyporheic biogeochemical processes are essential for mediating the chemical quality of adjacent water compartments (Boulton et al., 1998; Janauer, 2000).

One of the major questions concerning the role of hyporheic zones is how and to what extent biodiversity contributes to the riverine ecosystem functioning and resilience. The general hypothesis is that biodiversity contributes positively to ecosystem processes and represents an insurance against environmental variations and disturbances (Loreau et al., 2001). The benthic compartment of rivers provides habitats for many species and communities, as such creating a specific ecosystem. The activities and biodiversity of benthic invertebrates closely connect to microbial functions and related biogeochemical processes in riverbeds. Bioturbation, as an inherent benthos activity directly influences the physical structure and consequently the biological and chemical nature of sediments. In fine sediments, biogeochemical processes dominated by microbial activity are tightly linked to macrofauna and meiofauna e.g. (1) particle and solute displacements driven by macrofauna (François et al., 2002; Gerino et al., 2003), (2) agglutination of detritus particles by mucus secretions or proteolytic capacity stimulated by meiofauna (e.g. Riemann & Helmke, 2002; Nascimento & Naslund, 2012). In macro-porous hyporheic sediments, where particle sizes are similar or larger than those of benthic organisms, bioturbation is mainly performed by biofilm consumers and galleries diggers that modify sediment porosity (Mermillod-Blondin et al., 2003; Mermillod-Blondin & Rosenberg, 2006; Nogaro et al., 2007). A change in porosity may thus influence (1) pore water flow and the associated solutes transport, (2) microbial metabolism pathways and intensities, and consequently (3) solutes uptake.

Nutrient cycling and organic matter transformation within the hyporheic zone is mediated mainly by microorganisms which account for over 90 % of the community respiration (Pusch, 1996). However, these microorganisms are under a top-down control by organisms of higher trophic level such as scraping or shredding invertebrates. So interactions between microbial and invertebrate communities should be considered as a controlling factor for biochemical processes (Nogaro et al., 2008). Furthermore, the diversity of invertebrates could also drive these processes (and thus the self-purification capacity of hyporheic zone) (Nogaro et al., 2007). Influences of cross-community interactions (i.e. microorganisms-meiofauna-macrofauna) have been studied in ecosystemic description of energy fluxes and trophic webs by *in situ* investigations in autotrophic biofilms (Majdi et al., 2012b). Nevertheless, still few well controlled experiments in the literature are explore the effects of this biodiversity interrelation on ecosystem function e.g. excessive N load transformation and organic matter degradation (Webb & Montagna, 1993; Lillebø et al., 1999; Marshall & Hall, 2004) in heterotrophic biofilms.

The objective of the paper is to characterize the impact of biodiversity and cross-community efficiency on the ecological processes at the subsurface - surface water. Specifically, this study will consist in characterizing the role of cross-communities (biofilm, meiofauna, macrofauna) diversity on the uptake of nitrates and dissolved organic carbon by hyporheic sediments.

4.4 Materials and Methods

The methodology implemented here relies on laboratory experimentation through the use of microcosms i.e. sediment columns columns with water circulation to mimic a river hyporheic ecosystem. To test the role of biodiversity on nitrogen and carbon uptake rates, analysis of these elements were performed in water flowing through a series of microcosms reproducing a portion of water-sediment interface. The effects of community combinations in microcosms were tested by comparison of several experimental conditions setting a gradient of increasing community diversity.

4.4.1 Microcosm design

The microcosm design was following our previous study as described in (Sánchez Pérez et al., 2009), with some modifications 20 Plexiglas columns (height: 20 cm, internal diameter: 6.8 cm) were independently connected to water tanks to form 20 experimental units or microcosms (Fig. 4-1a). Abiotic sediment columns were filled with sand and gravel in four successive layers i.e. 0.5-1 mm, 1-2 mm, 2-10 mm and 10-20 mm (thickness: 2 cm). Each gravel and sand layer was sieved manually with the corresponding mesh before being autoclaved (20 min at 121 °C). This macroporous sediment structure (> 75 μm) allows fast solute transports. The total mass of sediment in each microcosm was 1000 ± 50 g. Mean porosity was 34 ± 3 %. A 300 μm filter was placed at the exit and entrance of the microcosm to maintain the sediment in the column. Silicone tubes (internal diameter = 3.2 mm) were used for connection to a high-density polyethylene tank with 15 l filtered water (90 μm) from the Garonne River (France). The water was collected several days before the beginning of the experiment in the Garonne River on April 2008. Peristaltic pumps (323Du Watson Marlow) were responsible for downward water circulation in microcosms, realizing a constant infiltration flow rate of 7-8 ml min^{-1} (Darcian velocity = 1.39-1.59 m d^{-1}) similar to the *in situ* range of water flow in hyporheic sediments (Weng et al., 2003; Peyrard et al., 2008). Supplied water was aerated in tanks to maintain oxygen saturation. All the microcosm-setups

(n = 20) were placed in a dark room to avoid phototrophic biofilm development. Room temperature was fixed at 15 ± 0.5 °C.

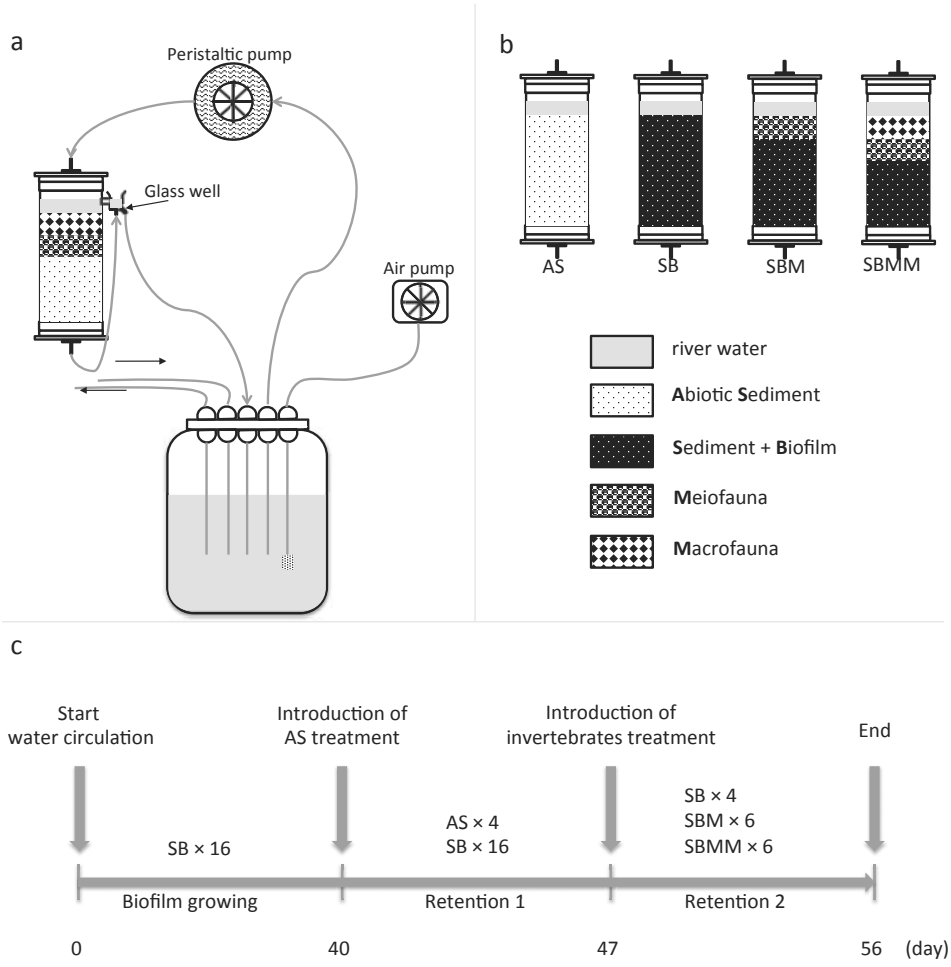

Figure 4-1 Microcosm design (a), treatment setup (b) and experimental design (c). Capital characters in bold were used to name the different treatment (b), i.e. AS = Abiotic Sediment, SB = abiotic Sediment + Biofilm, SBM = abiotic Sediment + Biofilm + Meiofauna, and SBMM = abiotic Sediment + Biofilm + Meiofauna + Macrofauna

4.4.2 Experimental design

Treatment setup – The experimental design is shown in Fig. 4-1b. Four different biodiversity levels were set in the microcosms to allow comparison of their functioning: abiotic sediment (AS); sediment and biofilm (SB); sediment, biofilm and meiofauna (SBM); sediment, biofilm, meiofauna and macrofauna community assemblage that correspond to the total benthic community of a river bed sediment (SBMM). Water circulation was activated in a total of 16 microcosms. After 40 days of incubation, these microcosms were assigned to SB. Another 4 microcosms were activated as started then as AS, to enable to evaluate the biofilm effect (AS × 4 *vs* SB × 16) during a 7-day period (Retention 1). Sediment and water for AS were autoclaved just before the beginning of water circulation to limit biofilm

development in these microcosms. At day 47, 16 SB microcosms were divided into three treatments i.e. SB (n = 4), SBM (n = 6) with miofauna added and SBMM (n = 6) with macrofauna added. Retention 2 period was used to compare biodiversity effect and lasted for 7 days.

Biofilm incubation – For the treatments with biofilm, the experiment lasted 90 days. To provide nutrients for constant biofilm growth, KNO_3 and $CH_3COONa \cdot 3H_2O$ were added to each tank and adjusted to the final concentrations ($N\text{-}NO_3^-$, 10 mg L^{-1}, DOC, 30 mg L^{-1}) once a week.

Invertebrate sampling and microcosm colonization – In situ invertebrate communities were collected in the Leze River (a sub-tributary of the Garonne River, South West France). Organisms, detritus and some sediment were collected with a "double net" surber equipped with a 55 and a 250 µm nets that make it able to sample meiofauna (55 – 250 µm) and macrofauna (> 250 µm) simultaneously. The three fractions (organisms, detritus and some sediment) were divided into subsamples of approximately the same fresh weight, and were introduced together at the top of the sediment into SBM and SBMM at the beginning of the experiment. A set of three additional subsamples of these three fractions was used for invertebrate identification and counting. Replicates of these subsamples were dried (121 °C during 3 hours) and introduced in all microcosms without invertebrate biodiversity to supply the same amount of sediment and organic matter as to the other microcosms. These meio and macro fauna inoculum subsamples weighed approximately 8 and 89 g (dry weight) respectively.

4.4.3 Experimental analysis

Biofilm biomass – The biomass of interstitial biofilm (including fauna when present) was determined at the end of the experiment by ash free dry mass (AFDM). A few grams (10 %) of sediment of each column taken at the top and bottom of the column were dried at 105 °C for 48 h and then burned off at 500 °C for 5 h. Ash free dry mass was calculated as the difference between the dry weight and the ash weight, to be used as a proxy of the biofilm biomass. The average of the two sediment samples was used for each microcosm.

Physic-chemical analysis – For nitrate concentration, water samples from the tank were filtered through cellulose acetate membranes (25 mm diameter, 0.2 µm and VWR) and analyzed by a high performance ion chromatographic analyser (DIONEX, DX500 and DX120). For dissolved organic carbon concentration, water samples were filtered (Whatman GF/F glass-fiber, 0.7 µm, 25 mm diameter, and pre-combusted at 500 °C for 4 h) and

acidified with concentrated hydrochloric acid (6N) until pH < 2 (10 µL HCl per ml of filtrate) and kept in 8 ml glass tubes (pre-combusted at 500 °C) in the refrigerator, then examined by a Total Organic Carbon Analyzer (Shimadzu TOC-5000A).

Meio/macro fauna identification – Three more replicates of wet subsamples with fresh invertebrates were stored at the initial time for fauna quantification. At the end of the experiment, 90 % of the total sediment in each microcosm of SBM and SBMM were used for identification and quantification of the remaining communities. Samples were preserved in 5% formalin until sorting of organisms. Meiofauna and macrofauna (Tachet et al., 2002) were identified at the lowest taxonomic level as possible using a stereo dissecting microscope.

Aerobic respiration and denitrification – Aerobic respiration and denitrification were measured at the end of the experiment following the slurry technique (Furutani et al., 1984). About 10 g of wet sediment of each sediment layer was placed in 150 mL flasks supplemented with a feeding solution in order to optimize microbial activity. For the measurements of N_2O production (denitrification), the incubation was under anaerobic conditions with a N_2 atmosphere. The feeding solution was a mixture of 5 mL of a KNO_3 (2.2 g L^{-1}), glucose (7.5 g L^{-1}) and glutamic acid (7.3 g L^{-1}) solution. For the measurements of CO_2 production (respiration), the incubation was realized under aerobiosis with 5 mL of a feeding solution of glucose (7.5 g L^{-1}) and glutamic acid (7.3 g L^{-1}). Then incubation flasks were filled with helium (He). The sequence was repeated three times, and inside pressure was adjusted to atmosphere. After removal of 15 ml of He from the incubation flasks, 15 mL of C_2H_2 (10% v/v final volume) was added to inhibit N_2O reductase. All incubations were carried out at 20 °C, in the dark and gently shaken. At 3 h and 6 h, gasses (C–CO_2 and N–NO_2) were measured by gas chromatography on a MTI 200 microcatharometer and dry weights of the sediment samples used were determined after drying at 60 °C to express the results as µg of C or N per hour and per gram of dry weight sediment (µg h^{-1} g^{-1} sed DW).

4.4.4 Nutrient uptake rates

The definition of nutrient uptake rate is refered to the total quantity of nutrient that is removed from water when passing through the sediment of microcosms, is estimated by the changes of concentrations over time in the reservoir water. In this paper, the uptake rate of nutrient is the quantification of the retention process. The differences in N-NO_3^- and DOC concentrations in the tank water between two sampling dates (time interval: 7 days) and the fresh weights of the sediment (sedFW) in each microcosm were used to calculate the uptake rates, which were finally expressed as “mg N or C.d^{-1}.kg^{-1} sedFW”.

4.4.5 Statistics

The equality of variances of the dataset was tested using Levene test. Log transformed dataset was used if the assumption was violated. For comparing certain variables in two treatments, a student t test or a Mann-whitney test were used depending on the equality of the sample sizes of the datasets. ANOVA test was used to analyze differences between three treatments. Tukey post-hoc test was used to determine the different groups.

4.5 Results

4.5.1 Macrofauna and meiofauna

At the end of the experiment, the mean ash free dry weight of sediments in SB and SBMM were 5.02 ± 0.39 g and 5.18 ± 0.43 g respectively, representing 0.5 % of organic matter in the microcosms. No differences were found among these treatments ($p > 0.05$).

A total of 29 macrofaunal taxa were introduced into SBMM. The total macrofaunal density was ranging from 191 to 380 individuals per microcosm. Diptera (Chiromidaes) dominated the macrofaunal community i.e. contributed 70 %, followed by Plecopteres (12 %), Coleoptera (5 %), Oligochaeta (4 %) and Hydrachnidiae (4 %) and a few Ephemeroptera (2 %) and Tricoptera (1 %). The dominant functional groups of macroinvertebrate at the initial period were scrapers (23 %), deposit feeders (22 %), shredders (20 %), predators (20 %), followed by filter feeders (9 %) and parasites (4 %). Total density per microcosm at the end of the experiments (48 ± 18 ind. per microcosm) was lower than that at the beginning of the macrofauna introduction (267 ± 25 ind. per microcosm). Taxonomic composition varied from the beginning compared with the end of experiments. The dominated taxa were Diptera (40 %), followed by Oligochaeta (29 %), Hydracarien (14 %) and Copepoda (8 %) of the total density of macrofauna at the end of the experiment. Predators became the most numeric functional feeding group (50 %) and followed by deposit feeder (26 %), Scraper (14 %), absorber (4 %) and shredder (3 %) of the total density at the end.

A total of 19 meiofaunal taxa were introduced into SBM and SBMM. The mean meiofaunal density at the beginning was 35296 ± 3956 ind. per microcosm. With a relative abundance of 84 % in both SBM and SBMM, rotifers were the most abundant organisms introduced in the microcosms with the meiofauna fraction, followed by Tardigrades (8 %) and meiobenthic Chironomidae larve (3 %). Total density per microcosm at the end of the experiments (5437 ± 3596 ind. per microcosm in SBMM and 5268 ± 2062 ind. per

microcosm in SBM) was lower than that at the beginning of the invertebrate introduction. Rotifers became even more dominant (95 % in SBM, 96 % in SBMM).

4.5.2 O_2 concentrations

At the end of Retention 1, mean O_2 concentration in SB was significantly lower than that in AS indicating a notable biofilm consumption of O_2 (Fig. 4-2). Significantly lower mean O_2 concentration was found in SBMM than in SB and SBM in Retention 2, highlighting the introduction of increased O_2 consumption.

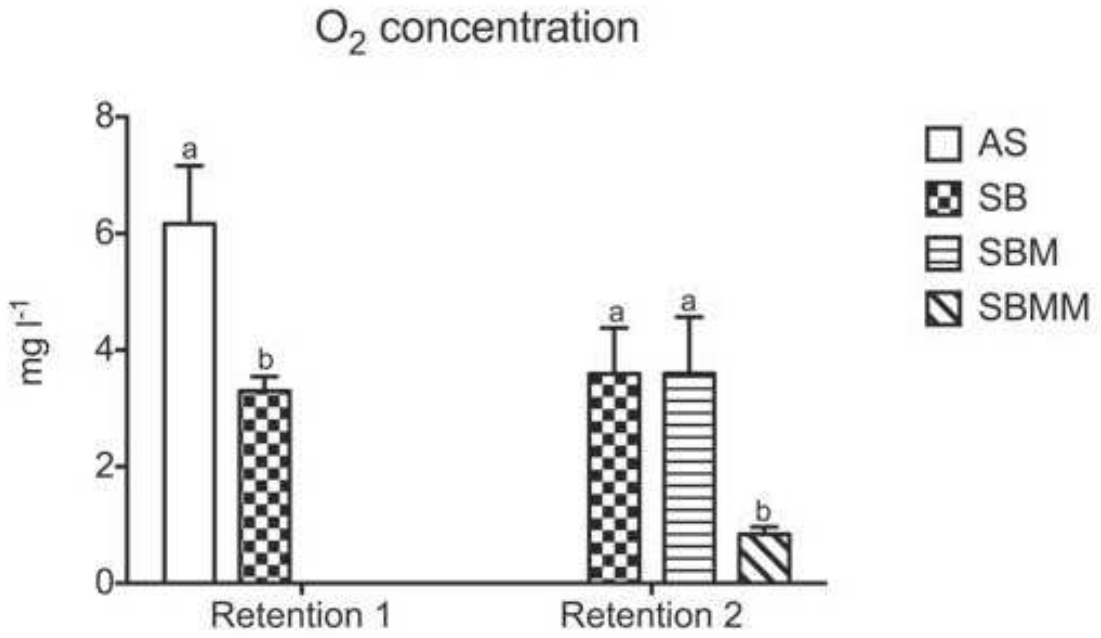

Figure 4-2 Oxygen concentrations at the end of the experiment (mean ± SE). Sample numbers are n = 4 for AS, n = 16 for SB in Retention 1, and n = 4 for SB, n = 6 for SBM, and n = 6 for SBMM in Retention 2. Different characters ("a", "b") resulting from statistic tests mark the treatments with significantly differences.

4.5.3 $N–NO_3^-$ and DOC uptake rates

At water circulation starting i.e. before nutrient enrichment and invertebrate addition, no differences in concentrations of $N–NO_3^-$ and DOC between treatments were found ($p > 0.05$). Mean concentrations measured in all microcosms were equal to 3.7 ± 1.0 mg L^{-1} for DOC and 1.8 ± 0.1 mg L^{-1} for $N–NO_3^-$. At the start of Retention 1 i.e. after addition of KNO_3 and CH_3COONa in each microcosm, mean concentrations of 31.2 ± 2.1 mg L^{-1} for DOC and 11.2 ± 0.5 mg L^{-1} for $N–NO_3^-$ were detected with no significant differences between treatments ($p > 0.05$).

In Retention 1, $N–NO_3^-$ uptake rate in SB was significantly higher than that in AS indicating a positive hyphorheic biofilm effect (Fig. 4-3a). $N–NO_3^-$uptake rates in SB did not change with time ($p > 0.05$), indicating a stable ability of mature biofilm for $N–NO_3^-$uptake.

However, in Retention 2, with the introduction of meiofauna, $N–NO_3^-$ uptake rate was increasing significantly i.e. SBM > SB, and the addition of macrofauna resulted in the significantly highest $N–NO_3^-$ uptake rate – 8.92 ± 0.69 mg N d^{-1} kg^{-1} sedFW compared to the other treatments. It is implied that the increasing invertebrate diversity enhanced the efficiency of $N–NO_3^-$ uptake in the microcosms.

In Retention 1, DOC uptake rate in SB was significantly higher than that in AS implying a positive hyphorheic biofilm effect, as on $N–NO_3^-$ uptake (Fig. 4-3b). Similarly, mean DOC uptake rates in SB did not vary in Retention 2 compared with uptake rates in Retention 1 ($p > 0.05$). However, in Retention 2, mean of DOC uptake rates in SBM was 51.00 ± 1.39 mg C d^{-1} kg^{-1} sedFW, significantly higher than the uptake rates in SB. Besides, DOC uptake in SBM was also significantly higher than that in SBMM.

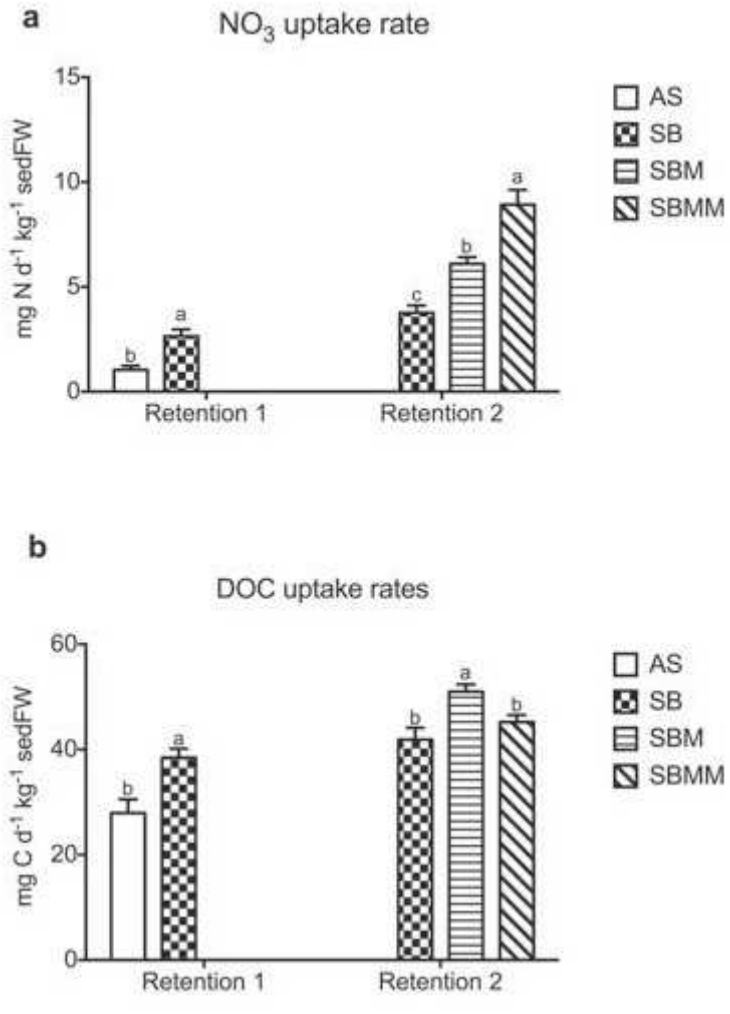

Figure 4-3 NO_3^- uptake rates (a) and DOC uptake rates (b) at the end of the experiment (mean ± SE). Sample numbers are n = 4 for AS, n = 16 for SB in Retention 1, and n = 4 for SB, n = 6 for SBM, and n = 6 for SBMM in Retention 2. Different characters ("a", "b") resulting from statistic tests mark the treatments with significantly differences.

4.5.4 Microbial activities

Mean denitrification rate in SBMM was significantly higher (6-fold) than that in SB (Fig. 4-4a) No significant difference in mean respiration rate between SBMM and SB (Fig. 4-4b), was found.

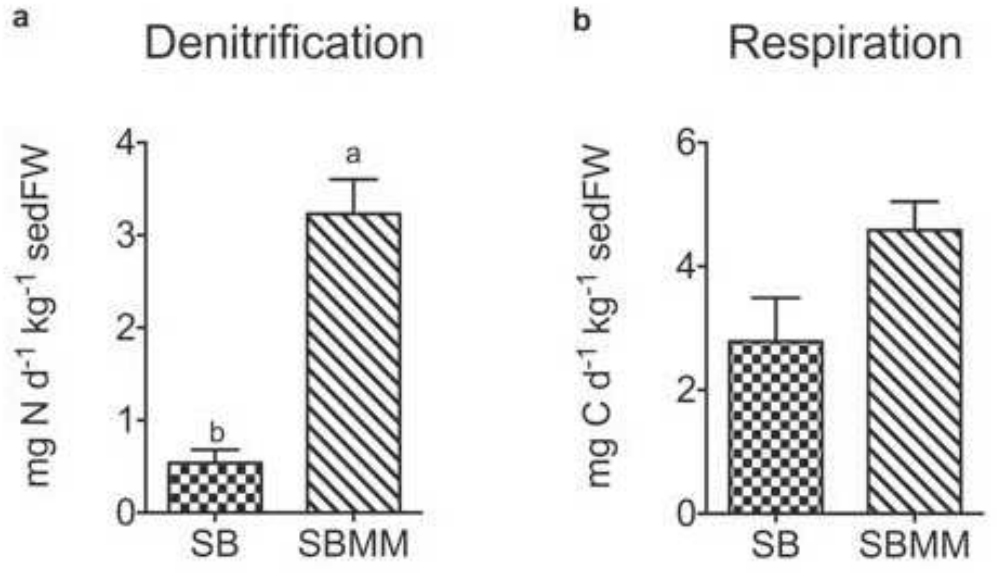

Figure 4-4 Denitrification (a) and respiration (b) rates at the end of experiment (mean ± SE). Sample numbers are n = 4 for SB, n = 6 for SBMM. Different characters ("a", "b") resulting from statistic tests mark the treatments with significantly differences.

4.6 Discussion

4.6.1 Hyporheic biofilm effect on nitrate and DOC uptakes

Our results show notable nitrate and DOC uptakes in the treatment with only sterilized sediment and recirculating river water, suggesting that processes going on during the 7-day early biofilm development can remove nitrate and DOC from the water phase. Droppo et al. (2007), studying biofilm growing in freshwater sediments, found that young biofilm (5 day age) was dominated by bacteria, and bacteria biomass represented over 50 % within 15 days of cultivation. Thus, heterotrophic bacteria may be the early settlers in the hyporheic zone. Previous studies suggest that in hyporheic biofilms, the ability of bacteria to acquire inorganic N is responsible for the early nitrate and DOC uptake in hyporheic zones (Findlay et al., 2003; Findlay & Sinsabaugh, 2003).

Since early biofilm development happened in AS, the comparison of uptake rates between AS and SB is no longer considers the absence and presence of biofilm, but the early (7 days age) and mature (56 day age) biofilms. In our experiments, nitrate and DOC uptakes significantly increased with biofilm age. Change in such uptake efficiencies achieved by the

heterotrophic consortium may be due to changes of biofilm biomass, 3-dimensional configuration of biofilm and/or its species composition or populations activity levels. Among these biological factors, biofilm thickness and therefore its biomass is one of major parameters that influences biofilm functioning and consequently water quality (Sabater et al., 2002; Battin et al., 2008). Although data on biofilm biomass evolution with time is not provided by the present study, it is assumed that the biofilm biomass increased with time. It can be thus envisaged that when biofilm biomass increases in hyporheic zones, steep redox gradients may occur and anoxic zones may be created where anaerobic pathways take place in deeper layers, thus redox gradients could be one of the explanations for nitrate and DOC cyclings. These redox gradients may be successful for the cycling of nitrate and DOC (Nielsen et al., 1990; Triska et al., 1993; Claret, 1998b).

4.6.2 Invertebrate community effect on nitrate and DOC uptake

Our results recorded a remarkable diversity effect on nitrate uptake rates i.e. with increasing biodiversity level. Nitrate uptake efficiencies were enhanced with additional invertebrate communities compared to single biofilm treatment (SBMM > SBM > SB > AS, Fig. 4-3a). This demonstrated not only the influence of biodiversity but also the positive effect of interaction between invertebrates and biofilm which we here call cross-communities effects. This is, to the best of our knowledge, is the first demonstration of such biodiversity effect at the level of the communities on water quality in hyporheic ecosystem.

The one-fold higher nitrate uptake rate in SBM than that in SB indicated the role of the meiofaunal group in stimulating the nitrate removal process. Unfortunately, the denitrification rate in SBM is not available.

Meiofauna i.e. benthic lotic rotifers – the most abundant in our microcosms – are primarily microphagous i.e. consuming microalgae, bacteria, protozoan and/or yeast (Ricci & Balsamo, 2000; Duggan, 2001; Mialet et al., 2013). Thus, their effect on uptake rates could be also interpreted as partly resulting from the meiofauna feeding activity that could change the microbial flora and/or stimulate the microbial growth (e.g. Aller & Aller, 1992; Liu et al., 2015). Bonaglia et al. (2014) showed how the presence of nematodes (without macrofauna) can increase nitrate removal efficiency from marine sediments through enhancing bacterial denitrification rate, which provides a rationale that meiofauna can stimulate the growth of denitrifying bacteria. Consequently, we can suggest that the higher nitrate uptake rate in the presence of meiofauna could indirectly result from the bioturbation activity of rotifers, stimulating N- treating bacteria.

The taxa composition of macrofauna varied from the beginning to the end of the experiments, however, Diptera were dominant throughout the experiment. Also a notable increase of Oligochaete density percentage was recorded in the composition, which may due to their tolerance to the effect of nutrient loadings (Giere, 1980; Verdonschot, 1996). The decrease in macrofauna density during the experiment may result in part from the high fraction of predators at the end of the experiment. The meiofauna also showed a decrease of total density during the experiment with increasing rotifer dominance in the community. This probably resulted from the experimental condition impossibly from top-down effect of macrofauna. It is known that rotifers are resistant to perturbed environment (Palmer et al., 1992; Majdi et al., 2012a).

The two-fold higher nitrate uptake rate in SBMM than that in SB implied that macrofaunal organisms can facilitate the self-depuration process in hyporheic zones. It may be emphasized that among the treatments in our experiments, SBMM could reflect the *in-situ* condition of a river bed. Thus, comparison of N uptake with and without macrofauna indicates that the lack of either macrofauna, or meiofauna could result in negative effect on nitrate removal efficiency by biofilm. This demonstration may be useful as argument for invertebrate biodiversity conservation by indicating this complete biodiversity, with possible cross community interactions, is a prerequisite for self-purification service efficiency. Our results show that, not only macrofauna, but also meiofauna are involved in this service performance, i.e. can indirectly interfere with the relative efficiency of biofilm to improve water quality. Diptera larvae, dominant in our experiments, are known as being characteristic of one mode of bioturbation i.e. bioirrigation which refers to the process of benthic organisms flushing their burrows with overlying water (Roskosch et al., 2010). This results in the exchange of dissolved nutrients e.g. nitrate between running water and sediments (Ford et al., 1999; Michaud et al., 2006). Thus, the contribution of macrofauna such as Diptera could be the one of the main accelerators of nitrate uptake in hyporheic zones. Nitrate uptake rates increase between SBM and SBMM also includes the possibility of the interactions between macro- and meiofauna communities. Few studies provide the influence of such interactions on nitrogen concentration changes in aquatic ecosystems. Recently, Bonaglia et al. (2014) pointed out that, in the presence of macrofauna (bivalves), high meiofauna densities (mainly nematodes) do not stimulate denitrification, which contrasts with our findings that denitrification rate was higher in SBMM than SB. This underlies the need to understand this type of interactions to better estimate their impact on nitrate uptake in ecosystems.

Unlike for nitrate uptake rate, our results showed that higher DOC uptake rates occurred in SBM than in both SB and SBMM microcosms. No differences in DOC uptake were observed between SB and SBMM. This shows that the meiofauna activity stimulated heterotrophic bacterial activity compared to the one taking place in microbial mats only, but this stimulation was less effective when meio and macrofauna communities were combined (SBMM). It is very likely that the potential increase of bacteria growth stimulated by meiofauna inputs was responsible for the higher DOC uptake since heterotrophic bacteria use DOC as a carbon source. Macrofauna has been reported to decrease both meiofauna activity and abundance in marine sediments due to disturbance, predation or competition for food (Alongi, 1985; Branch & Pringle, 1987; Ólafsson et al., 1999; Bonaglia et al., 2014). Besides, in running waters it is known that macrofauna can affect nitrate uptake ability of phototrophic biofilms negatively by reducing their biomass (Sabater et al., 2002). It is thus possible that the observed negative effect of macrofauna was due to (1) the predation on meiofauna which could limit the growth and activity of meiofauna, and further indirectly the bacterial DOC uptake, and (2), by consuming the biofilm biomass. Moreover, the equivalent respiration rates in SB and SBMM supported the assumption that the bacteria activity is limited by the addition of macro and meiofauna community. Michaud et al. (2006) reported a concomitant increase in nitrate and DOC uptake rates with the presence of macrofaunal gallery-diffusor, however, the biodiffusors had much less effect on DOC flux. This suggests that the effect of macrofauna on DOC uptake is probably related to the functional groups i.e. the modes of bioturbation (Michaud et al., 2005).

Most of the previous studies of invertebrates–microbial community interactions in biofilms underlined the macrofaunal effects on nutrient uptakes rates effects with a negative relation: macro-consumers might substantially depress the global biomass of the biofilm, and therefore the final outcome of the element cycling (Mulholland et al., 1994; Sabater et al., 2002; Marshall & Hall, 2004). The fact that we measured a positive relation suggests that the interaction may occur through other pathways e.g. stimulating the growth of bacteria (Liu et al., 2015) which could counterbalance the biomass reducing effect. The major possible effect of biodiversity that explains the increase of metabolism and its efficiency is then likely the results of cross-compartment interactions.

4.6.3 Comparison with *in situ* nitrate uptake

In our study, the nitrate uptake rates in all treatments were calibrated by microcosm area to allow comparison with *in situ* investigations. The present results ranged from 0.10 to

2.34 mg N m^{-2} min^{-1}, averaged 0.91 ± 0.10 mg N m^{-2} min^{-1} (mean ± SE, calibrated by microcosm area), which fall in the range of those measured in 11 European rivers i.e. from 0.11 to 11.0 mg N m^{-2} min^{-1} and averaged 1.94 ± 0.31 mg N m^{-2} min^{-1} (n = 65, unpublished data, J.M Yao et al.). This suggests that our microcosms quite successfully mimicked a natural river bed scenario and reflected a real nutrient uptake capacity of the hyporheic zone. This is an opportunity to underline that the fauna effect is inherently included in all *in situ* nitrate uptake measurements.

4.6.4 Conclusion

This study aimed to emphasis the important roles of biodiversity on biogeochemical (nitrogen and carbon) uptake efficiencies in subsurface–surface water interface. This study shows that for nitrate uptake rates especially, microbial diversity, meiofauna and macrofauna diversity in hyporheic biofilm is favouring the efficiency of this natural service. This observation confirms that cross-community diversity effect plays a role in the self-purifying service, and it should be considered with the same attention as the intra-community diversity effect. This study also provides a demonstration of that a loss of biodiversity might threaten ecosystem's functioning (Loreau, 2000; Loreau et al., 2001; Petchey, 2004). Recent studies, indeed, have suggested that the biodiversity decrease might reduce ecosystems' services through feedback mechanisms (Worm et al., 2006), with potentially important socio-economic consequences (Costanza et al., 1997). Also, since this experiment demonstrates the influence of hyporheic sediment and related biodiversity on nutrient uptake, the preservation of hyporheic zone in rivers looks like a primary condition to develop this service in nature.

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Chapter 5: La biodiversité influence-t-elle les effets du diuron sur la consommation de l'azote, dans le milieu hyporhéique ?

5.1 Résumé de l'article

5.1.1 Contexte et objectifs

Les herbicides sont très utilisés en agriculture constituant 70% de l'ensemble des pesticides utilisés (e.g. Kellogg et al., 2002) et sont parmi les polluants les plus communs dans les cours d'eau. Parmi eux le diuron (N-[3,4-dichlorophenyl]- N,N-dimethylurea; CAS No. 330-54-1) dont la concentration observée dans les cours d'eau européens atteint 2.1 - 36 µg L^{-1} (López-Doval et al., 2009). Dans le Chapitre 4, il est suggéré qu'en milieu hyporhéique, la capacité de consommation des nitrates augmente avec le niveau de diversité et que les interactions macrofaune-méiofaune-bactéries jouent un rôle essentiel dans l'augmentation observée. Dans l'hypothèse pour laquelle le diuron serait capable de modifier le taux de consommation d'azote par le biofilm, l'objectif de cette étude est de déterminer si l'augmentation de la biodiversité, pourrait réduire l'effet du diuron sur ces processus dans le milieu hyporhéique? L'étude est basée sur le principe expérimental présenté dans le Chapitre 4 modifié par l'apport d'herbicide (diuron) dans les traitements suivants: sédiment abiotique (ST) ; sédiment + biofilm (SBT), sédiment + biofilm + méiofaune (SBMT), sédiment + biofilm + méiofaune + macrofaune (SBMMT), pendant une période de 7 jours (T = diuron).

5.1.2 Principaux résultats et discussion

La consommation des nitrates par les biofilms hétérotrophes est apparue significativement modifiée par l'exposition au diuron (effet d'intensification, SB < SBT). De plus, aucune différence significative des taux de consommation n'a été observée entre les microcosmes ayant reçu la diversité maximale d'invertébrés, avec et sans diuron (SBMM et SBMMT). Dans l'ensemble, il apparait donc que la présence des invertébrés ait réduit l'effet (d'intensification) du diuron sur le taux de consommation de l'azote par les biofilms. Les invertébrés modifient les gradients physiques et chimiques des sédiments notamment par leur activité de bioturbation (Gerino et al., 2003; Mermillod-Blondin et al., 2003). Bien que les processus impliqués restent à élucider, cette étude conforte l'hypothèse du rôle potentiel essentiel que pourraient jouer les interactions macrofaune-méiofaune-micro-organismes dans la résistance des milieux hyporhéiques face aux perturbations chimiques,

Does biodiversity influence the effect of diuron on N uptake in the hyporheic zone?

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5.2 Abstract

The herbicide diuron is among the most common pollutants in rivers and can threaten the drinking water ecosystem service provision. The toxic effects of diuron on benthic invertebrates and microorganisms are also well-documented. Microbial biofilms, which are complex aggregates of microorganisms and their excretions grow at the water-sediment interface and through the hyporheic zone. This latter is recognised as a site of high biogeochemical activity that participates in stream eco-functioning by changing water quality. Invertebrates including macrofauna and meiofauna are ubiquitous and abundant in hyporheic biofilms, which acts as a patchy refugium for the invertebrates. Water quality favors biodiversity in ecosystems and inversely, biodiversity can have an impact on water quality through functional activities such as microbial metabolism, invertebrate community bioturbation and grazing. The present study aims to answer the following questions: (1) does the presence of diuron in the hyporheic zone change the N uptake by microorganisms? (2) Does cross-community biodiversity influence the effect of diuron on N uptake in this zone? Therefore, experimental microcosms were designed to realize different levels of hyporheic biodiversity (1. sediment only, 2. Sediment with biofilm, 3. Sediment with biofilm and meiofauna, 4. sediment with biofilm, meiofauna and macrofauna). The results show that

nitrate uptake rate increased with the addition of diuron in hyporheic zone, nevertheless, increased biodiversity i.e. addition of macrofauna and meiofauna, significantly reduced the stimulating effect of diuron on nitrate uptake. This enhanced ecosystem function is explained by bioturbation of invertebrates. This study suggests that under diuron exposure, the potential of the interactions between invertebrates and microorganisms could be considered as a main driver of the water quality amelioration process and highlights that the roles of meiofauna and macrofauna have yet been neglected.

Keywords:

Diuron; biodiversity; invertebrates; nutrient uptake; hyporheic zone; water-sediment interface; river bed

5.3 Introduction

Herbicides are widely used in agricultural management and account for 70 % of all agricultural pesticide use in the USA (e.g. Kellogg et al., 2002). They are among the most common pollutants in rivers and can reach these water courses via runoff from crop fields, spray drift, leaching, or accidental spills (Thurman et al., 1991). Diuron (N-[3,4-dichlorophenyl]- N,N-dimethylurea; CAS No. 330-54-1) is a broad-spectrum residual herbicide. The diuron concentration in European rivers reported in literature was in a range of 2.1 - 36 μg L^{-1} (López-Doval et al., 2009). Various experimental studies have been done to investigate the toxic effect of diuron on microorganisms, e.g. diuron can inhibit photosynthesis in algae and cyanobacteria by limiting the production of adenosine triphosphate (ATP) used for various metabolic processes (Corbett, 1984; Hayes, 1991; Pesce et al., 2006; Ricart et al., 2009). The toxic effects of diuron on freshwater invertebrates have also been documented. Diuron can cause mortality for certain species (especially *Lumbriculus variegatus* sp.) at high concentrations e.g. > 3.5 mg L^{-1} (Sanders & Cope, 1968; Nebeker & Schuytema, 1998). Diuron can disrupt endocrine activity in recombinant yeast assays at much lower concentrations (0.26 mg L^{-1}) (Noguerol et al., 2006).

In river ecosystem, microbial biofilms (a complex community of microorganisms and their excretions) grow at the water – sediment interface (phototrophic biofilm) (e.g. Sabater et al., 2002) and in the sediments of the hyporheic zone (e.g. Barlocher & Murdoch, 1989), an interphase zone between groundwater and the benthic area of rivers (Orghidan, 1959; 2010). The hyporheic zone is now recognized as a site of high biogeochemical activity (Pusch et al., 1998) which participates in stream eco-functioning by changing water quality (Stanford & Ward, 1993; White, 1993; Storey et al., 1999). Since the hyporheic biofilm is non-photosynthetic (i.e. heterotrophic), it might not be affected by diuron. However, the microbial metabolism that occurs in this biogenic structure may be able to interact with other molecules, like pesticides, and could thus play a role in the diuron removal process.

Invertebrates including macrofauna and meiofauna are ubiquitous in benthic environments and abundant in hyporheic biofilms (Boulton et al., 1998; Robertson, 2000). The hyporheic zone indeed acts as a patchy refugium for certain benthic invertebrate taxa (meiofauna: e.g. rotifers, copepods, cladocerans and harpacticoida and macrofauna: e.g. chironomids) during flood (Palmer et al., 1992; DoleOlivier et al., 1997).

Water quality favors biodiversity in ecosystems (e.g. De'ath & Fabricius, 2010) and inversely, biodiversity can have an impact on water quality through functional activities such

as microbial metabolism, bioturbation by invertebrate communities, and grazing (Hulot et al., 2000; Loreau, 2001; Lawrence et al., 2002; Timmermann et al., 2008). Cardinale (2011) has shown that, increasing algal species richness led to higher achieved nitrogen removal rates in lotic ecosystems, implying that biodiversity helps to buffer natural ecosystems against the ecological impacts of nutrient pollution. However, the role of biodiversity to participate in the bioremediative capacity applied to the pollutants is not clearly known, neither in rivers nor in the hyporheos (Gifford et al., 2007). We previously demonstrated that an increasing level of cross-community diversity could favor nitrogen and dissolved organic carbon (DOC) uptake by heterotrophic biofilm in the hyporheic zone (Liu et al., submitted). This is supported by the suggestion that the interaction between invertebrates and bacteria could participate in excessive N reduction (Bonaglia et al., 2014; Liu et al., 2015). Moreover, in hyporheic zones, engineering by invertebrates' bioturbation could enhance both the porosity of the sediments and the solute transport across the water- sediment interface, and stimulate microorganisms to utilize more dissolved organic matter and nutrients. (Gerino et al., 2003; Mermillod-Blondin et al., 2003).

The present study aims to answer the following questions: (1) does the presence of diuron in the hyporheic zone change the N uptake by microorganisms? (2) Does cross-community biodiversity influence the effect of diuron on N uptake in this zone? To answer these questions, experimental microcosms were designed to realize different levels of hyporheic biodiversity (1. sediment only, 2. sediment with biofilm, 3. sediment with biofilm and meiofauna, 4. sediment with biofilm, meiofauna and macrofauna) to study nutrient uptake from the interstitial water in presence of diuron and under different conditions of biodiversity.

5.4 Methods

This experiment was part of a large-scale setup of testing nitrate uptake capacity by hyporheic biofilms in the Inbioprocess project. A first set of results considering the effect of cross-community diversity on nitrate removal capacity was reported in a previous paper (Liu et al., submitted). The present paper considers an additional manipulation during which diuron was added in half of the microcosms composing the entire experiment. For clarity, the microcosm design is described below.

5.4.1 Microcosm design

The experiments were performed in sediment column microcosms with downward water circulation to mimic a river hyporheic ecosystem. The microcosm design was the same as described in (Sánchez Pérez et al., 2009) (Fig. 4-1a). 20 Plexiglas columns (height: 20 cm, internal diameter: 6.8 cm) were assembled and filled with successive 2 cm thickness layers of 4 different granulometric ranges of sand and gravel i.e. 0.5-1 mm, 1-2 mm, 2-10 mm and 10-20 mm. Each gravel and sand layer was sieved manually with corresponding mesh before being autoclaved (20 min at 121 °C). The total mass of sediment in each microcosm was 1000 ± 50 g fresh weight. Mean porosity was 34 ± 3 %. A 300 µm filter was placed at the exit of the microcosm to maintain the porosity. Silicone tubes were used for connection to a tank with 15 L filtered water (90 µm) from the Garonne River (France). The water was collected several days before the beginning of the experiment in April. Peristaltic pumps (323Du Watson Marlow) were used for water circulation in microcosms, realizing a constant infiltration flow rate of 7-8 ml min^{-1} (Darcian velocity = 1.39-1.59 m d^{-1}) similar to the *in situ* range of water flow in hyporheic sediments (Weng et al., 2003; Peyrard et al., 2008). Supplied water was aerated in tanks to maintain oxygen saturation. All the microcosm-setups (n = 30) were placed in a dark room to avoid phototrophic biofilm development. Room temperature was fixed at 15 ± 0.5 °C.

5.4.2 Experimental design

Treatment setup – In the present study, diuron concentration (CAS: 330-54-1, log Kow = 2.78) was set to 30 µg L^{-1} diluted in 0.1 % DMSO. This concentration was selected in order (1) to be able to measure this molecule during a long-term experiment taking into account a strong sediment adsorption effect and (2) to remain in the range of non-lethal

effects reported in literature. The diuron was added into all the tank water on day 56 of the experiment after biofilm and invertebrate settling. Microcosms were divided into four different conditions of hyporheic biodiversity (Fig. 5-1) which were labeled "T" indicating the presence of the herbicide: ST (abiotic sediment, n = 4), SBT (sediment and biofilm, n = 4), SBMT (sediment and biofilm with meiofauna addition, n = 6) and SBMMT (sediment and biofilm with meio/macro fauna additions, n = 6). For ST, the sediment and water was autoclaved just before the beginning of circulation of water with toxic, to limit biofilm development. For the other treatments, with biofilm, water circulation began two months before water circulation for ST in order to obtain a substantial biofilm biomass. Another two treatments without addition of diuron were set up simultaneously as the 'T' ones, in similar conditions, to monitor the heterotrophic biofilm and invertebrate effects without toxic, i.e. SB (sediment and biofilm without diuron, n = 4) and SBMM (sediment and biofilm with meio/macro fauna additions without diuron, n = 6). For the density and biodiversity of macrofauna and meiofauna, samples were taken twice i.e. before day 0 and after 75 days. Diuron was injected into treatments labeled "T" at 68 days, and the samples for diuron concentration measurement were taken at 75 days (Fig. 5-1a). In order to calculate nutrient retention, samples for measuring nitrate and DOC concentrations were taken twice i.e. at 66 days and at 75 days.

Biofilm incubation – For the treatments with biofilm, the experiment lasted 75 days. To provide nutrients for constant biofilm growth, KNO_3 and $CH_3COONa \cdot 3H_2O$ were added once a week to each tank and adjusted to the same final concentrations: $N\text{-}NO_3^-$, 10 mg L^{-1} and DOC, 30 mg L^{-1}. For ST, these nutrient inputs were started only one week before diuron addition to limit biofilm development.

Invertebrate sampling and microcosm colonization – In situ invertebrate communities were collected in the Leze River (a third order sub-tributary of the Garonne River, South West France). Meiofauna (55 – 250 μm) and macrofauna (> 250 μm) were collected separately with Surber nets according to the methods described in (Liu et al. submitted). Invertebrate additions to the microcosms were realized by adding sediments containing the living communities to SBMT, SBMM and SBMMT microcosms at day 46. Meanwhile, 8 and 89 g (in term of dry weight) were added for meiofauna and associated sediment and for macrofauna and associated sediments respectively. For ST, SB and SBT, the sediment samples were sterilized at 121 °C during 3 hours and introduced to supply the microcosms with the same amount of sediment and organic matter as to the other treatments.

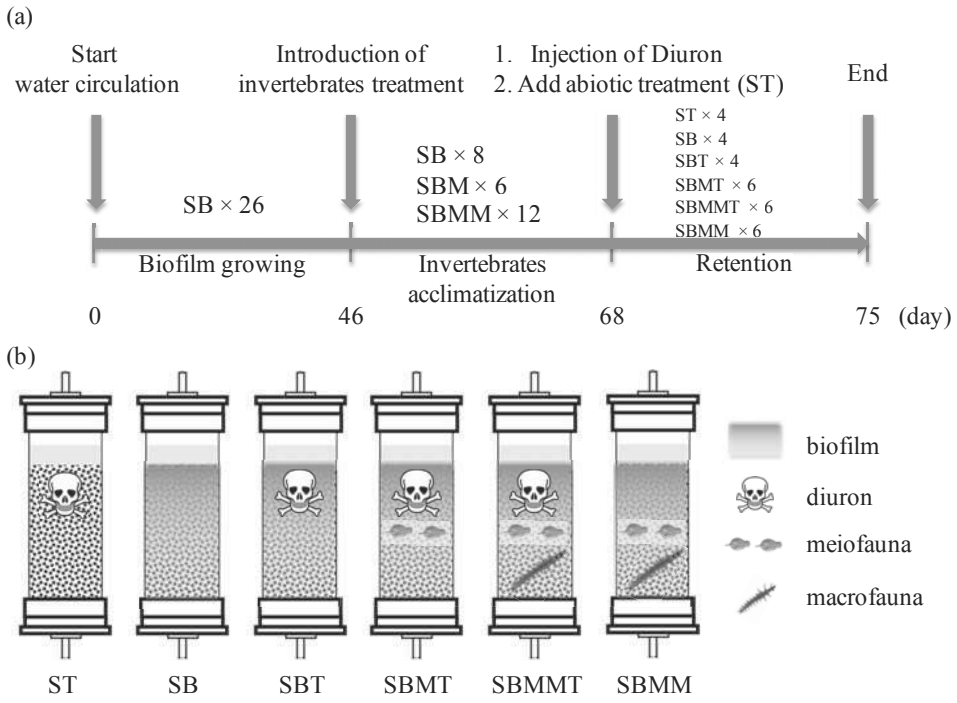

Figure 5-1 Experimental design (a) and treatment setup (b)

5.4.3 Experimental analysis

Organic matter content – was measured in the sediment at the end of the experiment as ash free dry mass (AFDM). A few grams (10 %) of sediment of each microcosm taken at the top and bottom of the sediment column were dried at 105 °C for 48 h and then burned off at 500 °C for 5 h.

Physico-chemical analysis – Nitrate concentration was analyzed by high performance ion chromatographic analyser (DIONEX, DX500 and DX120). DOC concentration was measured by Total Organic Carbon Analyzer (Shimadzu TOC-5000A). Details of the procedure are given in the previous paper (Liu et al. submitted). Diuron and its main metabolite i.e. 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU) in water were analyzed by ESI-LC-MS/MS (API 4000, Applied Biosystems) at the end of the experiment i.e. 18 d after injection.

Meio/macro fauna identification – Three more replicates of wet subsamples with fresh invertebrates were stored at the initial time for fauna quantification. At the end of the experiment, 90 % of the total sediment in each microcosm of SBMT, SBMM and SBMMT were used for identification and quantification of the remaining communities. Samples were preserved in 5 % formalin until sorting of organisms. Meiofauna and macrofauna (Tachet et al., 2002) were identified at the lowest taxonomic level as possible using a stereo dissecting

microscope. Biodiversity in the microcosm was expressed as taxa richness (Colwell, 2009) and by Shannon index (Shannon & Weaver, 1949).

Aerobic respiration and denitrification – Aerobic respiration and denitrification were measured at the end of the experiment following the slurry technique (Furutani et al., 1984). About 10 g of wet sediment of each sediment layer was placed in 150 mL flasks supplemented with a feeding solution in order to optimize microbial activity. For the measurements of N_2O production (denitrification), the incubation was under anaerobic conditions with a N_2 atmosphere. The feeding solution was a mixture of 5 mL of a KNO_3 (2.2 g L^{-1}), glucose (7.5 g L^{-1}) and glutamic acid (7.3 g L^{-1}) solution. For the measurements of CO_2 production (respiration), the incubation was realized under aerobiosis with 5 mL of a feeding solution of glucose (7.5 g L^{-1}) and glutamic acid (7.3 g L^{-1}). Then incubation flasks were filled with helium (He). The sequence was repeated three times, and inside pressure was adjusted to atmosphere. After removal of 15 mL of He from the incubation flasks, 15 mL of C_2H_2 (10% v/v final volume) was added to inhibit N_2O reductase. All incubations were carried out at 20 °C, in the dark and gently shaken. At 3 h and 6 h, gases ($C–CO_2$ and $N–NO_2$) were measured by gas chromatography model on a MTI 200 microcatharometer and dry weights of sediment were determined after drying at 60 °C to express the results as µg of C or N per hour and per gram of dry weight sediment (µg h^{-1} g^{-1} sed DW).

5.4.4 Nutrient uptake rates

For this experiment, we define nutrient uptake rate as the total quantity of nutrients that is removed from water when passing through the sediment of microcosms. It is estimated from the changes of concentrations over time in the reservoir water. In this paper, the uptake rate of nutrients is the quantification of the nutrient retention process. The differences in $N-NO_3^-$ and DOC concentrations in the tank water between two sampling dates (time interval: 7 days) and the fresh weights of the sediment (sedFW) in each microcosm were used to calculate the uptake rates, which were finally expressed as "mg N or C.d^{-1}.kg^{-1} sedFW".

5.4.5 Statistics

Student *t* test or Mann-Whitney test (one-tailed) was used depending on the equality of the sample sizes of the datasets to examine the differences in (1) the four variables (nitrate and DOC uptake rates, denitrification and respiration rates) between e.g. SB and SBT, SBMM and SBMMT respectively, and (2) invertebrate richness and Shannon index between

the beginning and the end of the experiment. One-way ANOVA was used to test the difference in ash-free dry mass of sediment in all treatments at the end of the experiment.

5.5 Results

5.5.1 Macrofauna and meiofauna

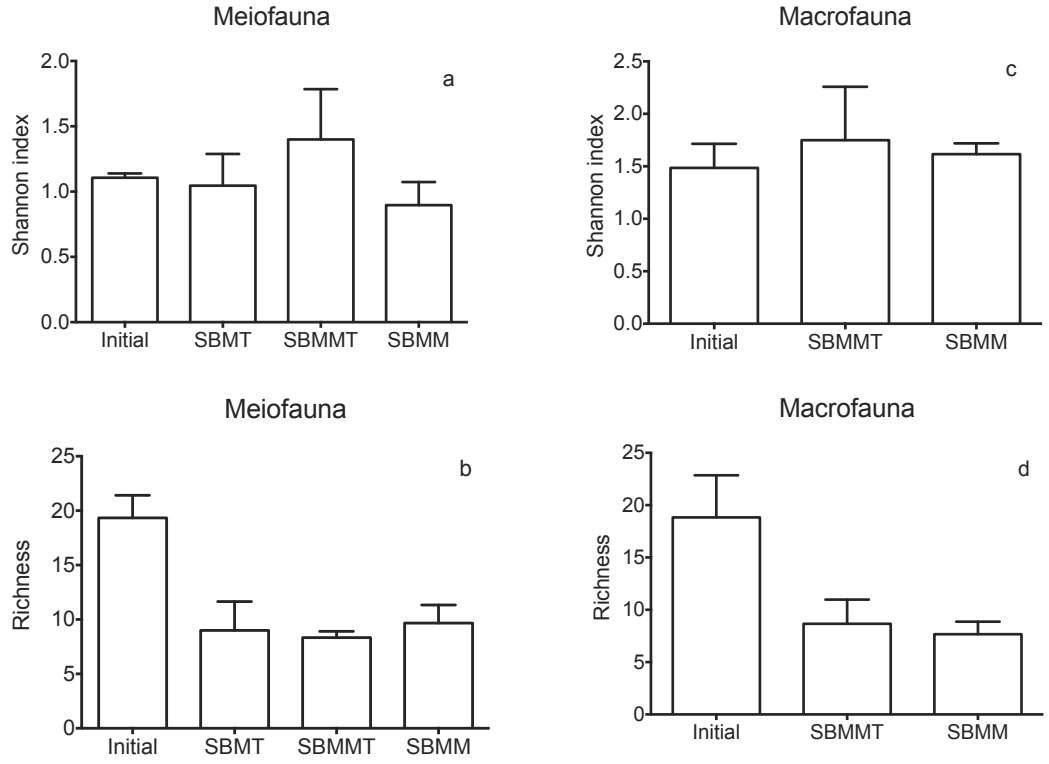

Figure 5-2 The Shannon index and richness of meiofauna and macrofauna (mean ± SD, n= 4 or 6) in microcosms before the water circulation (i.e. intial, 0 d) and after the addition of diuron i.e. at the end of this experiment (75 d). "Initial" treatment, n = 3, sediment with meiofauna and macrofauna; "After: SBMT", n = 3, sediment + biofilm + meiofauna + diuron; "After: SBMMT", n = 3, sediment + biofilm + meiofauna + macrofauna + diuron.

At the end of experiments, no differences were found for the ash free dry weight of sediments between all treatments (ANOVA, $p > 0.05$), with a mean of 5.31 ± 0.17 g (mean ± standard error, n = 12).

At day 46, a total of 29 taxonomic groups of macrofauna were introduced into SBMM and SBMMT treatments including Diptera (chironomidae), which dominated the macrofauna community and contributed 70 % of the total macrofauna density (ranging from 191 to 380 individuals per microcosm), followed by Plecopteres (12 %), Coleopteres (5 %), Oligochaetes (4 %) and Hydracariens (4 %) and a few Ephemeropteres (2 %) and Trichopteres (1 %). The dominant functional groups of macrofauna were scrapers (24 %), deposit feeders (22 %) and shredders (21 %), followed by predators (17 %), filter feeders (9

%) and parasites (5 %). At the end of the experiment, total macrofaunal density (48 ± 18 ind. per microcosm, n = 3) was lower than that at the beginning (267 ± 25 ind. per microcosm, n = 6). At the end of the experiment, the dominating taxonomic group was Diptera (40 %), followed by Oligochaetes (29 %), Hydracariens (14 %) and Copepods (8 %) relatively to the total density of macrofauna, and, scrapers became the most abundant functional feeding group (45 % of the total density.), followed by deposit feeders (21 %), predators (16 %) and shredders (14 %).

19 taxonomic groups of meiofauna were introduced into both SBMT and SBMMT treatments at the beginning of the experiment but rotifers were dominant with a relative density of 84 %, followed by Tardigrade (8 %) and chironomidae larvae (3 %). At the end of the experiment, in SBMT treatments, relative density of rotifers increased to 95 % followed by nematodes (5 %). In SBMMT treatments, rotifers and tardigrades accounted for 68 % and 30 % of total meiofauna densitiy at the end of the experiment.

No significant difference in taxa richness and Shannon index was observed between the treatments SBMM and SBMMT, showing that the presence of diuron did not affect the diversities of meiofauna and macrofauna.

5.5.2 Diuron and DCPMU concentrations

The diuron concentrations obtained at the end of the experiment (Fig. 5-3) range from 14.8 to 23.4 µg L^{-1} with an average concentration equal to 20.4 ± 1.9 µg L^{-1}. No significant difference in diuron concentrations was found between 1) ST and SBT, 2) SBMT and SBMMT respectively. However, diuron concentrations in SBMT and SBMMT were significantly lower than that in SBT indicating that invertebrates have a positive effect on diuron degradation.

DCPMU concentrations at the end of the experiment are shown in Fig. 5-3b. A significantly higher concentration of the metabolite was found in SBT than in ST ($P < 0.001$). It indicated that the Diuron-to-DCPMU degradation was higher in the treatments with biofilm. Contrasting with results observed for Diuron concentrations, there was no significant difference in DCPMU concentration between treatments with invertebrates (SBMT and SBMMT) and SBT, showing that the presence on invertebrates did not contribute to enhance the Diuron-to-DCPMU degradation in our microcosms.

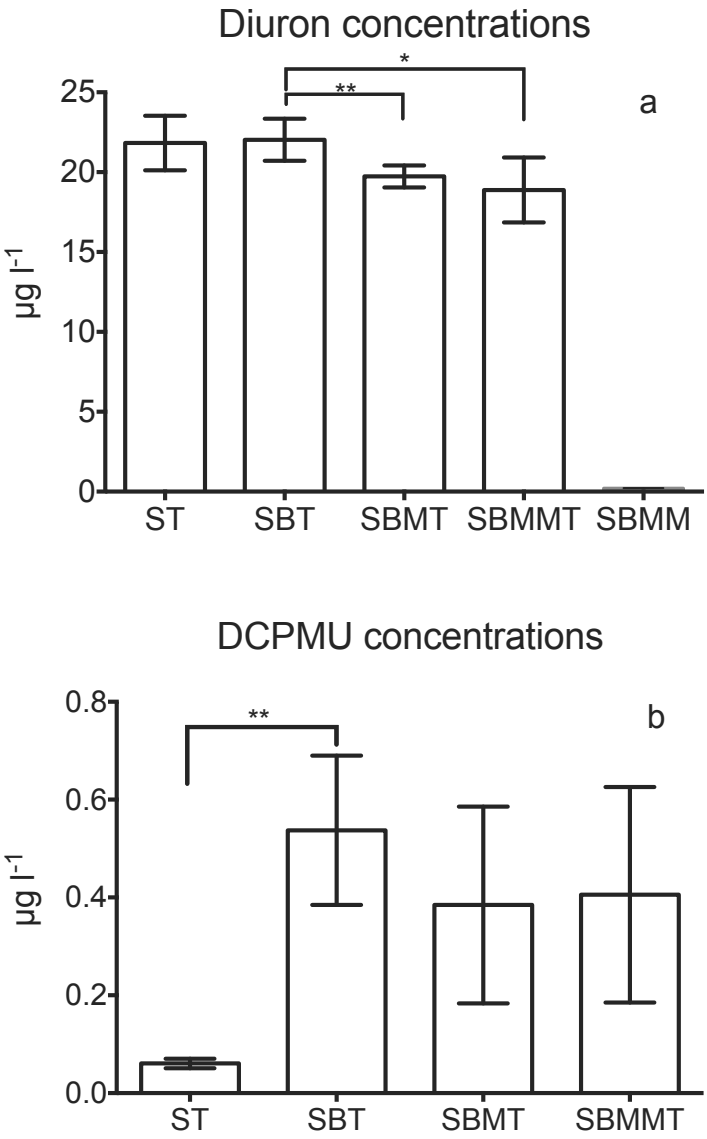

Figure 5-3 The concentrations of diuron (a) and DCPMU (b) (mean ± SD, n= 4 or 6) at the end of the experiment. "ST" treatment, n = 4, sediment + diuron; "SBT", n = 4, sediment + biofilm + diuron; "SBMT", n = 6, sediment + biofilm + meiofauna + diuron; "SBMMT", n = 6, sediment + biofilm + meiofauna + macrofauna + diuron; "SBMM", n = 2, sediment + biofilm + meiofauna + macrofauna. * and ** show significant differences when P < 0.05 and < 0.01 respectively (one-tailed t test).

5.5.3 Nitrate and DOC uptake rates

During the 7-day period of uptake measurement, the $N\text{-}NO_3$ uptake rate in SBT was significantly higher than that in SB ($P = 0.028$, Fig. 5-4a). This indicates – somewhat surprisingly- that the presence of diuron stimulated $N\text{-}NO_3$ uptake rates in sediments with biofilm. However, no significant difference of $N\text{-}NO_3$ uptake rate was found between SBMMT and SBMM treatment ($P > 0.05$). Among T treatments nitrate uptake rate measured in ST was significantly lower than those in the treatments with invertebrates i.e. SBMT and SBMMT respectively but not significantly lower than those in the treatment with only biofilm i.e. SBT (Fig. 5-4a). This result indicates that in the presence of diuron, the maximal

diversity is able to positively influence (i.e. biofilm alone was not efficient) an increase in N-NO_3 uptake rates.

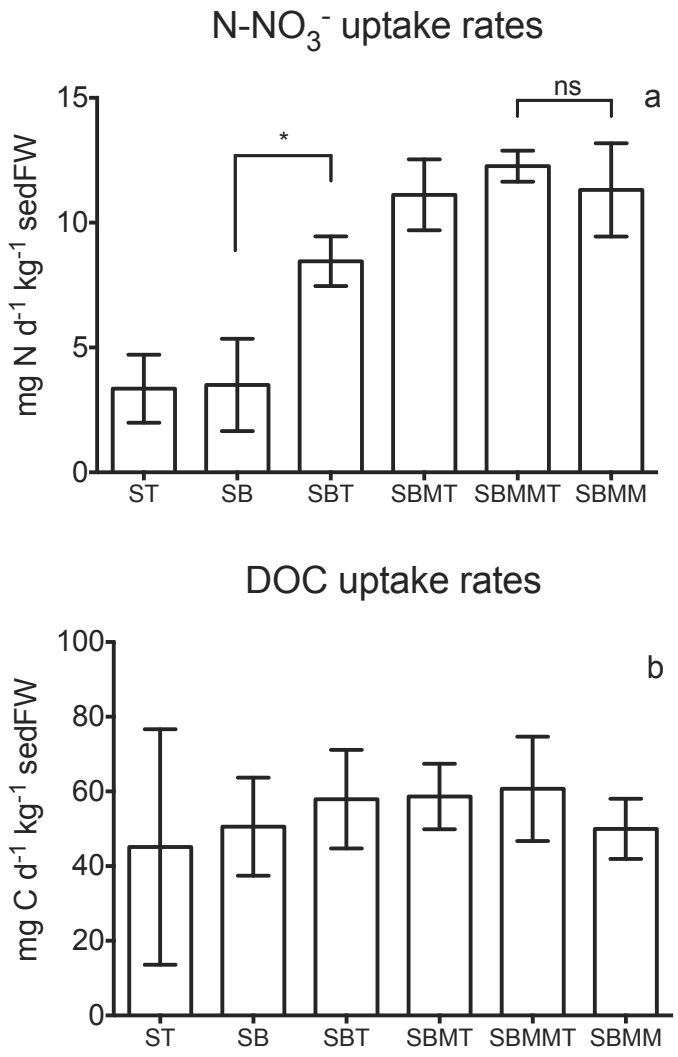

Figure 5-4 Nitrogen and DOC uptake rates (mean ± SD, n= 4 or 6) for seven days of the end experiment. "ST" treatment, n = 4, sediment + diuron; "SB", n = 4, sediment + biofilm; "SBT", n = 4, sediment + biofilm + diuron; "SBMT", n = 6, sediment + biofilm + meiofauna + diuron; "SBMMT", n = 6, sediment + biofilm + meiofauna + macrofauna + diuron; "SBMM", n = 6, sediment + biofilm + meiofauna + macrofauna. "a", "b" and "ab" show significant differences (ANOVA); "*" shows significant difference when P < 0.05, "ns" shows no significance for one-tailed t test between SB and SBT, SBMM and SBMMT.

During the 7-day period of uptake measurement, DOC uptake rates ranged from 45.11 ± 15.77 to 60.69 ± 5.71 mg C d^{-1} kg^{-1} sed FW. No significant differences were found between the treatments (Fig. 5-4b) showing that with or without the presence of diuron, the occurrence of invertebrate biodiversity did not modify the DOC uptake in the microcosms.

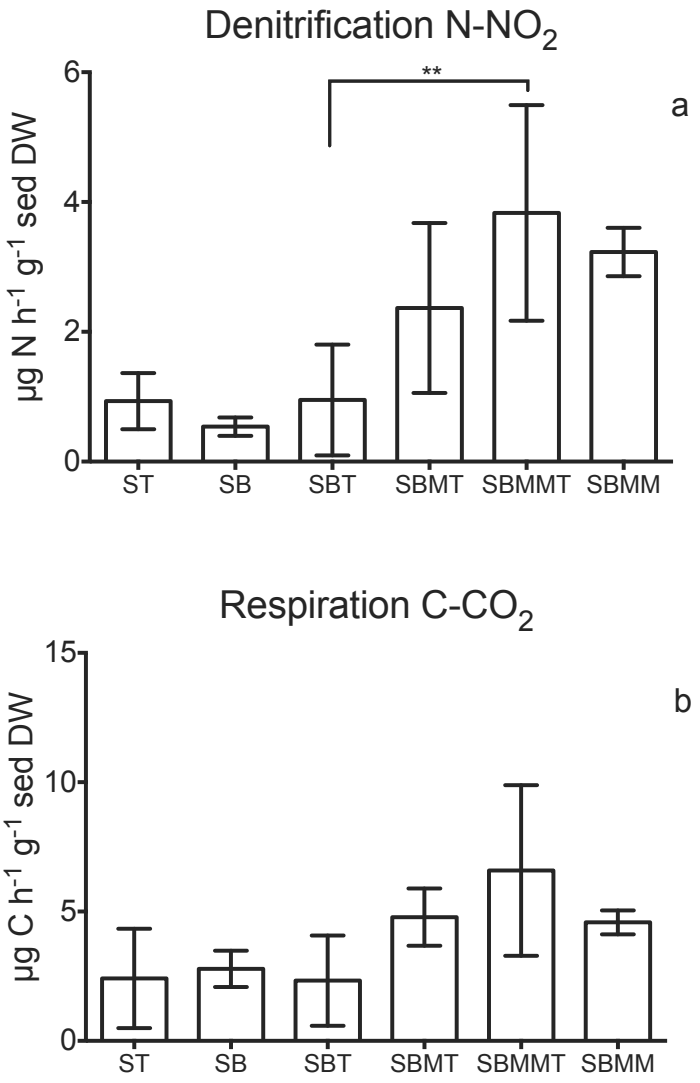

Figure 5-5 The productions of N_2O by denitrification and CO_2 by respiration processes (mean ± SD, n= 4 or 6) for seven days of the experiment. "ST" treatment, n = 4, sediment + diuron; "SB", n = 4, sediment + biofilm; "SBT", n = 4, sediment + biofilm + diuron; "SBMT", n = 6, sediment + biofilm + meiofauna + diuron; "SBMMT", n = 6, sediment + biofilm + meiofauna + macrofauna + diuron; "SBMM", n = 6, sediment + biofilm + meiofauna + macrofauna. ** show significant differences when P < 0.01 (one-tailed t test).

5.5.4 Denitrification and respiration

Denitrification and respiration rates are shown in Fig. 5-5. For these two variables, no significant differences were found between the treatments with and without diuron (SB and SBT, SBMM and SBMMT respectively), indicating that no effect of diuron was observed on these processes. No significant differences were found in respiration and denitrification rates between ST and SBT suggesting that, despite the late circulation to prevent biofilm development applied to ST, these microcosms were also colonized by a substantial biofilm biomass. Significantly higher denitrification rates were found in SBMMT than the rates measured in SBT. This result implies that the addition of invertebrate communities

contributed to enhance the denitrification potentialities in the microcosms. Although there was no significant difference in denitrification and respiration rates between SBMMT and SBMT, denitrification rates in SBMMT tended to be higher, suggesting that meiofauna occurrence by itself may not be sufficient to stimulate the biofilm metabolism. Only the addition of the macrofauna community, with all possible cross community interactions with meiofauna and microfauna in the microcosms, significantly increased respiration and denitrification rates.

5.6 Discussion

Biofilms typically dominate microbial life in ecosystems with high sediment-surface-area to water volume ratios. Generally, in the hyporheic zone, microbial processes exert control over net heterotrophy, and biofilms are the main ecosystem engineers supporting water quality improvement. These biofilms are usually considered as the main contributors to nutrient cycling in aquatic ecosystems (Battin et al., 2003; 2008). In nitrate-rich Thames estuary, the major part of nitrate uptake was due to heterotrophic bacteria, since the addition of an anti-biotic (a prokaryotic inhibitor) lowered uptake rates by 66 % (Middelburg & Nieuwenhuize, 2000).

As meio- and macrofuana are likely to influence the microbial activity within the hyporheic zones, it was interesting to test the effect of diuron on the biodiversity in our microscosms with and without diuron. Our results showed no effect of diuron on meio' or macrofauna or diversity. These communities are apparently resistant to diuron at the concentrations used in our experiment.

Secondly, as to the $N\text{-}NO_3$ uptake rates, significantly higher nitrate uptake rates were found in SBT than that in SB, indicating that nitrate uptake rate could increase with the addition of diuron in the hyporheic zone. As the mechanism behind such a stimulating effect is still unclear, further investigations are needed. We can, at present, only consider this increase as a perturbation of the normal $N\text{-}NO_3$ uptake mechanisms by hyporheic sediments. Nevertheless, when exposed to diuron, increased biodiversity i.e. addition of meiofauna and macrofauna, could significantly reduce the stimulating effect of diuron on nitrate uptake since there was no significant difference between $N\text{-}NO_3$ uptake rates in SBMM and SBMMT (Fig. 5-4a). This suggests that the hyporheic biofilm with higher biodiversity i.e. with invertebrates compared to the one without invertebrates could reveal enhanced resistance and/or resilience to the effect (increased $N\text{-}NO_3$ uptake rates observed between SBT and SB) of herbicide input.

Our previous results in the same experimental conditions demonstrated that, in the absence of diuron, invertebrates could enhance nitrate uptake rates (Liu et al., submitted). Here, our results show that under diuron exposure, besides the above described effect, we observed only a slight (but not significant) increase of N uptake rates with meiofauna addition (SBMT) compared to biofilm treatment (SBT). Significantly higher $N\text{-}NO_3$ uptake rates were recorded in SBMMT compared to SBT. The presence of biofilm together with both meio- and macro invertebrates (SBMMT), was needed to observe a significant increase in nitrate uptake as compared to SBT (Fig. 5-4a). In sediments, macrofauna, through different types of bioturbation activity (a review by Covich et al., 1999) can create changes in the direction and rates of flow, differential deposition of sediment grain sizes and organisms. Also, their burrowing and sediment reworking activity can change chemical gradients and dissolved oxygen concentration within the interstitial water. Colwell (1998) emphasizes that such "biocomplexity" of habitats and biological relationships is an important aspect of biodiversity. Bioturbation could create extensive biocomplexicty (Charbonneau & Hare, 1998), which could act not only as a buffer for nutrient cycling (Covich et al., 1999), but also influence ecosystem functioning through complex biogeochemical interactions (Lohrer et al., 2004; Caliman et al., 2007).

Bacterial denitrification is one of the pathways considered as biological nitrate uptake, and denitrifying bacteria are present and active in hyporheic zones (Sobczak et al., 2003; Pinay et al., 2009). In the aquifer of the Garonne River, sediment-attached bacteria exhibited denitrification ability (Iribar et al., 2007). In our experiment, no difference was found in denitrification rates between SB and SBT, suggesting that diuron did not affect denitrifying bacterial activity. Bonaglia et al. (2014) observed that in marine sediment, meiofauna bioturbation stimulate denitrifying bacteria, however, high densities of meiofauna in the presence of macrofauna do not stimulate denitrification, while the rate of dissimilatory nitrate reduction to ammonium is significantly enhanced.

The roles of meiofauna and macrofauna in nitrate uptake processes should attract more attention, especially meiofauna since they dominate in term of abundance in environments such as hyporheic zone and are till now less studied then macrofuana (Schmid Araya, 2000). The mechanisms that drive the cross-community interaction between the two invertebrate communities affects N uptake are still uncertain. Recent research suggests that predator richness can have cascading effects on communities and ecosystem properties, and the decrease in predator richness could lead to increase densities of prey (Bruno & Cardinale, 2008). In the present study, the richnesses of macrofauna and meiofauna were both decreased

during a 7-day period, thus the interactions among macrofauna, meiofauna and bacteria are difficult to predict. Even though, Hunter et al (2012) examined interaction of macrofauna and heterotrophs in low-oxygen sediment, and suggested that macrofauna could regulate bacterial activity potentially via complex niche-partitioning and interaction with other faunal group including meiofauna. Thus, we emphase that such interaction deserve further exploration.

It should be finally remarked that, in our experiments, $N\text{-}NO_3$ uptake rates but yet increased with biofilm only (i.e. in SBT compared with ST). It could be due to the early-developed hyporheic biofilm in ST and indicating that this younger biofilm (7-day age) presented similar effects on N uptake as the pre-existed biofilm (75 day age) in SBT. It is contrasted with our previous study that showed the early-developed hyporheic biofilm (7-day age) presented not similar but lower N uptake ability compared to the 47-day-age biofilm. No significant difference was found in N uptake rates between the two conditions with 7-day-age biofilm both in the present and previous study (in present study with diuron: 3.35 ± 2.73 mg N d^{-1} kg^{-1} sed FW; in previous study without diuron: 1.04 ± 0.38 mg N d^{-1} kg^{-1} sed FW), indicating no effect of diuron on the N uptake ability of young biofilm. Thus, in the present study, lack of response of the 75-day-age biofilm comparing to the 7-day-age biofilm to N uptake cannot be attributed to the diuron exposure but to the potential decreased microbial activity of the 75-day-age biofilm comparing to he 47-day-age biofilm.

Pesce et al. (2006) used microcosm experiments involving whole microbial communities (periphytic and planktonic) from a natural river with diuron concentration set at 10 μg L^{-1}. They found that bacterial production in diuron-treated samples was equal to, or significantly lower than in the control, indicating a potentially lower bacterial activity under diuron exposition. Proia et al., (2011) from laboratory grown phototrophic biofilms, suggested that diuron did not affected heterotroph microorganisms and thus, bacterial production was not limited by diuron addition. Similarly, in the present study, no significant positive or negative effect of diuron on DOC uptake was found, suggesting, that at the concentrations used in our experiment, heterotrophic bacteria metabolic function was not limited by Diuron toxicity at the present concentrations. Besides, some bacteria (e.g. *Variovorax* sp. and *Arthrobacter* sp.) can mineralize diuron to CO_2 (Sørensen et al., 2008) and it is known that a mixture of diuron and glyphosate can stimulate cell growth in co-culture of *Arthrobacter* sp. and *Delftia acidovorans* (Bazot & Lebeau, 2007). Sumpono et al (2003) found that the abundance of bacteria increased for two weeks after diuron introduction (10 mg L^{-1}) in lab-scale wastewater treatment ponds. Our results show that the mean diuron degradation rate was 21.9 % in ST and SBT indicating the occurrence of bacteria capable to

degrade diuron molecules. (Liu et al., 2010) showed that the burrowing activity of soil invertebrates (earthworm) could stimulate abundance and activity of herbicide degraders endogenous to soil. Thus, it is assumed that invertebrates may participate in the diuron degradation process in hyporheic zone.

5.7 Conclusion

This study mainly suggests that invertebrate community can reduce the effect of diuron on nitrate uptake rate by heterotrophic biofilms in the lotic hyporheic zones, and bioturbation is responsible for this enhanced ecosystem function. It is also shown that, also under diuron exposure, nitrate uptake capacity of hyporheic zones can be promoted by increasing levels of cross-community diversity. The potential of the interactions between invertebrates and microorganisms could be considered as a main driver of the water quality amelioration process. The role of meiofauna and macrofauna, so far rather neglected (Schratzberger, 2012) in improving water quality, is highlighted here by their indirect effect on N uptake.

Chapter 6: General discussion and conclusion

6.1 Version française

Ce travail a pour originalité de démontrer que les rotifères méiobenthiques, lorsqu'ils sont présents en densités élevées, peuvent contribuer à améliorer la capacité de consommation de l'azote, des biofilms phototrophes (Chapitres 2 et 3) et des biofilms hétérotrophes (Chapitre 4) de cours d'eau. Cela implique que la méiofaune peut jouer un rôle significatif sur les échanges en azote entre la colonne d'eau, le milieu benthique et la zone hyporhéique. Cet effet de la méiofaune est observé pour des concentrations en $N\text{-}NO_3$ relativement élevées (2,03 ± 0,04 mg L^{-1} et 2,7 ± 0,11 mg L^{-1} dans le biofilm phototrophe, Chapitres 2 et 3) simulant les conditions eutrophes de la zone aval de la Garonne. Il est aussi significatif pour des concentrations plus élevées (9,34 ± 1,19 mgL^{-1} dans le biofilm hétérotrophe, Chapitre 4) pouvant être notamment retrouvées en zone hyporhéique qui peut, sous certaines conditions, présenter des concentrations en nitrate supérieures à celles des eaux de surface (e.g. Krause et al., 2013). De plus, les résultats du Chapitre 5 indiquent que l'introduction d'invertébrés peut aussi diminuer la perturbation causée par un herbicide (le diuron) qui se manifeste par une stimulation de la consommation des nitrates par le biofilm lorsqu'il est exposé à cette molécule.

Suite à un enrichissement, la réponse des rotifères et des bactéries s'est traduite à court-terme par une augmentation concomitante (Chapitre 2) voire par une corrélation hautement significative (Fig. 3-3 et 3-4, Chapitre 3) de leur densité dans le biofilm. De plus, le Chapitre 4 suggère l'existence d'un lien entre la densité des invertébrés et des taux élevés de dénitrification des biofilms hétérotrophes. Dans leur ensemble, ces résultats suggèrent fortement que l'effet positif exercé par les rotifères sur le taux de consommation de l'azote ($N\text{-}NO_3$) résulterait d'interactions entre les rotifères et les bactéries des biofilms, en zones benthique et hyporhéique. Etant donné que différents types d'interactions peuvent lier bactéries et méiofaune (relations trophiques et indirectes, voir discussion du Chapitre 3), il apparaît nécessaire d'examiner leur implication respective dans ces processus.

L'effet de la méiofaune sur la consommation de l'azote par le biofilm a été observée sous conditions hydrodynamiques stables dans les études présentes. La question de l'extrapolation de ces résultats en milieu naturel se pose donc. Premièrement, les concentrations en $N\text{-}NO_3$ utilisées sont dans l'intervalle des concentrations rapportées pour la

Garonne (Iribar et al., 2007; Majdi et al., 2012a). Deuxièmement, les rotifères dominent souvent la densité de la méiofaune associée au biofilm comme montré pour la Garonne, pendant les périodes d'étiage (e.g. 2009/08/04 – 2009/10/04, Majdi et al. 2012a) où le biofilm et la méiofaune se développent, (Fig. 6-1). Ces deux indications permettent de suggérer que le développement de cette méiofaune associée au biofilm pourrait aussi influencer la consommation de l'azote par les biofilms en milieu lotique naturel. Cependant, il doit être noté que les densités de rotifères utilisées sont largement supérieures à celles observées sur le terrain (e.g. en moyenne 5.4 ± 1.3 10^3 ind cm^{-2} dans le chapitre 3 contre 0.06 ± 0.03 10^3 ind cm^{-2} dans Majdi et al. 2012a). L'extrapolation de ces résultats au milieu naturel doit donc être considérée avec prudence.

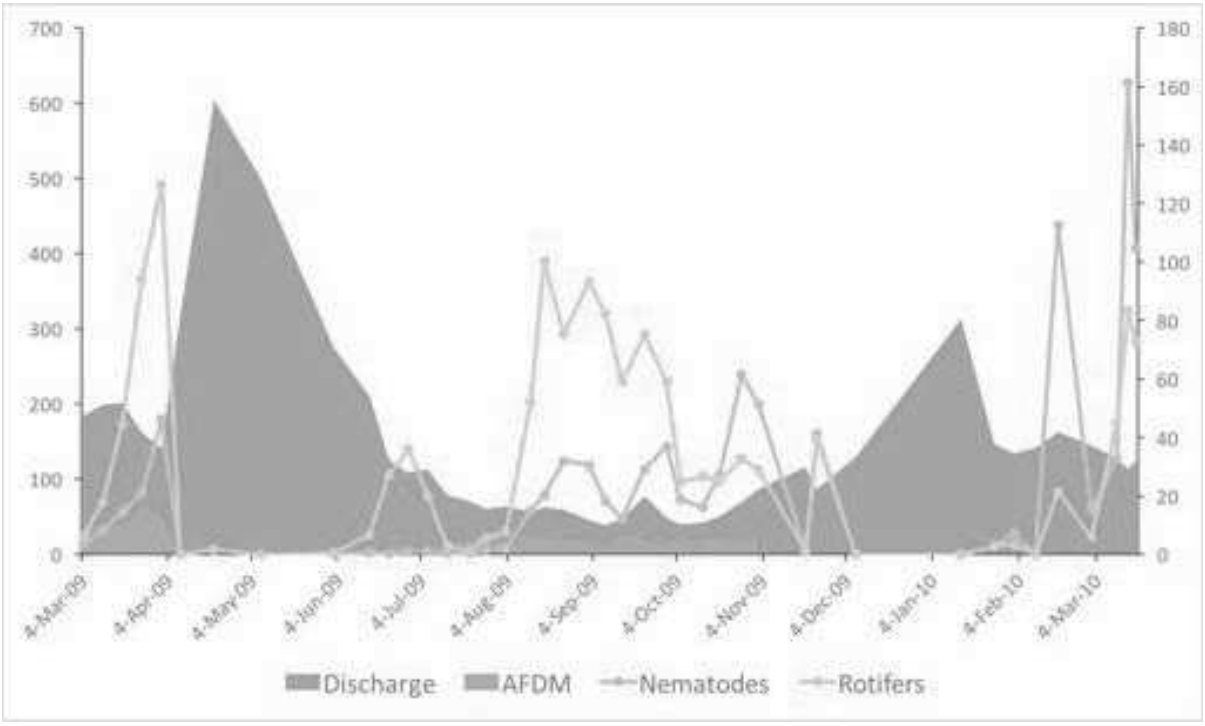

Figure 6-1 The densities of nematodes and rotifers (ind. cm^{-2}), discharge (m^3 s^{-1}) and biofilm biomass (AFDM g m^{-2}) in Garonne River during a period from 2009/03/04 to 2010/03/04 after Majdi et al. 2012a

Aucune variation significative concernant les micro-algues (analyses pigmentaires), n'a été observée, en réponse à l'enrichissement en N-NO_3 donc, aucune preuve de l'implication potentielle de la fraction microalgale des biofilms dans les processus de consommation des nitrates n'est apportée par ce travail. Cela peut paraître surprenant en particulier pour les biofilms phototrophes qui par définition, ont une large part de biomasse photosynthétique (e.g. Barranguet et al., 2005). Cette absence de réponse pourrait être due aux concentrations ambiantes utilisées dans les microcosmes enrichis, supérieures au seuil limitant la croissance

des algues benthiques d'eau douce (Stevenson et al., 1996). Cependant, il est aussi possible que la production primaire soit consommée par le méiobenthos, maintenant ainsi la biomasse microalgale à un niveau stable. Les rotifères sont en effet des consommateurs actifs au sein des biofilms (Mialet et al., 2013). La pression de « broutage » exercée par la méiofaune peut stimuler la croissance bactérienne (Traunspurger et al., 1997; Hakenkamp & Morin, 2000), donc, il peut être supposé que celle des microalgues puisse aussi être stimulée par le *grazing* du méiobenthos. Cela suggèrerait donc que ces interactions méiofaune- microalgues aient pu contribuer à l'augmentation de la consommation des nitrates par le biofilm. Par ailleurs, l'étude de Proia et al. (2012) suggère que face à un enrichissement en nutriments, les microalgues de biofilms épilithiques pourraient accroître leurs interactions avec les bactéries alors que leur biomasse n'augmente pas de façon significative en 52 jours. Dans la présente étude, l'activité des microalgues n'a pas été mesurée et donc, la question d'une possible implication des interactions méiofaune - bactéries - microalgues sur la capacité de consommation de l'azote par les biofilms subsiste. Un premier indice pourrait toutefois être apporté par la comparaison des accroissements des taux de consommation de l'azote ($N\text{-}NO_3$), par les biofilms phototrophes (Chapitre 3) et les biofilms hétérotrophes (Chapitre 4), obtenus dans les microcosmes enrichis en méiofaune.

Table 6-1 Comparaison des paramètres expérimentaux et des résultats issus des études des Chapitres 3 et 4. $N\text{-}NO_3$: concentration initiale moyenne (± SD) utilisée dans les microcosmes enrichis. Accroissement (%) : différence entre les taux de consommation ($N\text{-}NO_3$) moyens mesurés pour les biofilms à basse et haute densités de rotifères* (Chapitre 3), ou, non enrichis et enrichis en méiofaune (Chapitre 4).**

	Chapitre 3	Chapitre 4
Biofilms	Phototrophes (benthiques)	Hétérotrophes (hyporhéiques)
Substrats	Plexiglas	Sédiments
Lumière	Oui	Non
Température (°C)	17	15
Débit (mL min^{-1})	2500	7,5
Incubation (jours)	5	7
$N\text{-}NO_3$ (mg L^{-1})	2,70 ± 0,11	9,34 ± 1,19
Accroissement (%)	58,2	60,5
Méiofaune (10^3 ind. cm^{-2} ± SD)	5,4 ± 1,3*	1,0± 0,2**

L'accroissement du taux de consommation moyen du biofilm phototrophe après 2 jours d'exposition à l'enrichissement en $N\text{-}NO_3$ (concentration initiale de 2,70 ± 0,11 mg L^{-1}) est de 58,2% dans les microcosmes enrichis en méiofaune comparés à ceux qui n'ont pas été enrichis. Pour les biofilms hétérotrophes, 7 jours après une exposition à une concentration

initiale de 9,34 ± 1,19 mg L^{-1}, l'accroissement observé est de 60,5% (Tableau 6-1). Donc l'accroissement n'est pas différent pour les biofilms phototrophes comparés aux biofilms hétérotrophes. Les conditions expérimentales étaient différentes (Tableau 6-1), il faut donc considérer cette observation avec prudence : les biofilms phototrophes (Chapitre 3) ont été exposés à une température plus élevée bien que la concentration initiale en $N\text{-}NO_3$ et le temps d'incubation étaient inférieurs à ceux utilisés pour l'étude du chapitre 4 (biofilms hétérotrophes). Néanmoins, cette comparaison ne supporte pas la suggestion émise plus haut. Selon les résultats rapportés par Stock et al. (2014) relatifs à une incubation de sept jours et demi en microcosmes, les microalgues (diatomées) seules n'ont pas influencé les flux d'azote de sédiments marins en microcosmes tandis qu'elles ont provoqué une augmentation de l'effet positif de la méiofaune (copépodes) sur la réduction dissimilatrice des nitrates. De plus, les produits d'excrétion de la méiofaune peuvent favoriser la croissance bactérienne (e.g. Riemann & Helmke, 2002), donc, selon ces auteurs, les interactions entre la méiofaune, les algues et les bactéries pourraient être mises en jeu sachant que les microalgues pourraient indirectement augmenter la croissance bactérienne en influençant la qualité et la quantité des produits d'excrétion de la méiofaune, indirectement, à travers sa consommation de diatomées (Stock et al., 2014).

Les études des Chapitres 4 et 5 sur le milieu hyporhéique, intègrent la macrofaune benthique en plus de la méiofaune et du biofilm afin de tester différentes conditions de diversité. Elles ont montré que lorsque la diversité augmente, les taux de consommation d'azote ($N\text{-}NO_3$) et du COD augmentent aussi. Il est à noter que l'accroissement de la consommation de l'azote correspondant à l'introduction de la méiofaune dans les microcosmes, a encore été accru par l'introduction de la macrofaune (essentiellement des larves de Chironomidea) dans l'étude n'utilisant pas d'herbicide (Chapitre 4). La macrofaune peut avoir un effet de réduction de l'activité et de l'abondance de la méiofaune en raison de son activité de bioturbation et de prédation, et, par compétition pour les ressources trophiques (Alongi, 1985; Branch & Pringle, 1987; Olafsson, 2003). Par exemple, Bonaglia et al. (2014) ont montré que la présence du bivalve *Macoma balthica* contrebalance l'effet positif de la méiofaune sur la communauté microbienne associée aux processus de nitrification et dénitrification de sédiments marins. Selon les auteurs, cet effet résulte probablement d'interactions de compétition entre le bivalve et la méiofaune pour la matière organique fraîchement sédimentée, comme suggéré auparavant par Ólafsson et al., (2005) et Nascimento et al. (2011). Les résultats présentés dans le Chapitre 4 contrastent avec ces observations. Il faut considérer que les chironomides appartenant à l'endofaune, par leur

activité fouisseuse, agissent comme des organismes ingénieurs dans les écosystèmes d'eau douce (Stief, 2013). Ils pratiquent par exemple, une irrigation intermittente de leur galeries et ainsi stimulent les processus de nitrification et dénitrification sédimentaires dans les lacs (e.g. Pelegrí & Blackburn, 1996; Svensson, 1997; 1998; Lewandowski et al., 2007; Roskosch et al., 2010; Stief, 2013). Il a par ailleurs aussi été montré que la densité des bactéries associées au cycle de l'azote est plus élevée sur les parois des galeries de la macrofaune endogée que dans les sédiments environnants (Satoh et al., 2007). Il peut donc être envisagé que pendant la période expérimentale, les chironomides aient agi sur le taux de consommation d'azote par leur effet stimulant à la fois sur le couplage nitrification- dénitrification et sur la croissance bactérienne avec de probables conséquences sur les interactions bactéries – méiofaune (Chapitre 4). Ces résultats soulignent la nécessité d'approfondir l'étude des interactions existant entre la macrofaune, la méiofaune et les micro-organismes et de leur influence sur les flux d'azote à l'interface eau-sédiment sous-étudiée dans le passé (Bonaglia et al., 2014), en particulier, pour la compréhension des processus régulant la capacité de consommation des nitrates par les biofilms.

La mise en évidence de l'effet positif des rotifères sur la capacité de consommation des nitrates par les biofilms est supportée par leur réponse potentielle rapide suite à un enrichissement en nutriments (Chapitre 2). Cette observation concorde avec de précédentes observations de réponses de rotifères méiobenthiques soumis à des concentrations croissantes en phosphore, en habitats lacustres et en microcosmes (Särkkä, 1992; Ristau & Traunspurger, 2011; Ristau et al., 2012; Wu et al., 2014). Il est donc possible de suggérer que les assemblages de rotifères méiobenthiques pourraient jouer un rôle dans l'indication du niveau trophique des écosystèmes aquatiques comme cela a déjà été suggéré pour les rotifères planctoniques d'écosystèmes lacustres (Duggan et al., 2001).

L'implication des interactions rotifères-bactéries est fortement suggérée par la quasi-totalité des études expérimentales menées ici, à la fois pour les biofilms de surface (Chapitres 2 et 3) et dans le milieu hyporhéique (Chapitre 4). Aucune réponse à court terme liée à l'enrichissement en nutriments n'a été observée pour les nématodes. Cette absence de réaction est très probablement liée à leur très faible représentation dans ces études (0,3-10 ind. cm^{-2} dans le Chapitre 2, 0,2 % dans le Chapitre 3, et 1,7 % dans le Chapitre 4) bien qu'ils puissent aussi dominer la méiofaune associée aux biofilms e.g. 20-319 ind. cm^{-2} dans le biofilm de la Garonne (Majdi et al., 2012a). Gaudes et al. (2012) indiquent que la densité des nématodes de sédiments sableux de cours d'eau de forêts méditerranéennes, peut être positivement influencée par l'addition de nutriments, au cours d'une étude expérimentale de

2 ans. Cela suggère que les nématodes puissent réagir plus lentement à un apport en nutriments que les rotifères. Majdi et al. (2012a) ont montré que la croissance de l'assemblage des nématodes dans le biofilm épilithique de la Garonne est plus lente que celle des rotifères qui constituent le taxon « pionnier » suite à l'arrachage et au redéveloppement du biofilm dus aux crues. Il est connu par ailleurs, que les nématodes d'eau douce peuvent (1) consommer des bactéries et des algues (e.g. Moens et al., 2006), (2) stimuler la croissance bactérienne par leur production de mucus (Riemann & Helmke, 2002) et (3) modifier le cycle de l'oxygène et la production primaire de biofilms de diatomées (Mathieu et al., 2007). Il est donc probable que les nématodes influencent aussi les processus de consommation d'azote des biofilms, à travers leurs interactions avec les bactéries. Cette implication nécessite d'être établie au cours de futures investigations. Enfin, comme préalablement suggéré pour les sédiments marins par Bonaglia et al. (2014), il doit être noté que d'autres groupes associés aux biofilms et potentiellement actifs dans la régulation des processus impliquant la communauté microbienne, comme par exemple les eucaryotes unicellulaires hétérotrophes, sont encore sous-étudiés pour leur contribution au fonctionnement des biofilms lotiques.

Bien que la macrofaune puisse affecter la capacité de consommation des nutriments par les biofilms (e.g. Sabater et al., 2002), les résultats du travail présenté ici, soulignent qu'au contraire la méiofaune peut avoir un effet positif indirect sur cet aspect du fonctionnement des biofilms phototrophes et hétérotrophes, à travers ses interactions avec les micro-organismes et la macrofaune pouvant aussi réduire la perturbation du taux de consommation de $N\text{-}NO_3$ causée par l'introduction d'un herbicide dans le milieu. De plus, la réponse à court-terme des rotifères méiobenthiques face à un apport de nutriments couplée à leur meilleure résistance aux perturbations hydrologiques comparée à celle des nématodes (Majdi et al., 2012a) leur confère un intérêt particulier à la fois pour le développement d'indices d'évaluation de l'état écologique des écosystèmes lotiques et l'étude de procédés visant à améliorer la qualité des eaux. Ce potentiel a préalablement été montré pour les nématodes d'écosystèmes méditerranéens côtiers (Moreno et al., 2011) et de milieux estuariens (Patrício et al., 2012; Alves et al., 2013) mais ne semble pas encore avoir été étudié pour les rotifères des biofilms. Enfin, les résultats montrent aussi que la capacité « d'épuration naturelle » des biofilms de rivière peut être liée à leur diversité ce qui est en accord avec l'hypothèse générale qui considère que la biodiversité contribue positivement au fonctionnement des écosystèmes (Loreau et al., 2001).

Dans l'ensemble, ce travail met donc en évidence le rôle significatif que peut avoir la méiofaune dans les processus de consommation de l'azote par les biofilms lotiques. De plus,

les résultats suggèrent fortement que les invertébrés interagissent avec les micro-organismes impliqués dans les processus de réduction des concentrations en azote, dans le biofilm phototrophe comme dans le biofilm hyporhéique. Enfin, l'exposition à l'herbicide a engendré une modification significative du taux de consommation de $N\text{-}NO_3$ dans les microcosmes hyporhéiques. Cependant, la comparaison du taux de consommation moyen de $N\text{-}NO_3$ entre les traitements exposés à l'herbicide et ceux non exposés, a montré que la présence des invertébrés (méiofaune + macrobenthos) a significativement réduit l'effet du diuron sur ces processus. Cette étude met en exergue le rôle potentiellement important des interactions micro-organismes – invertébrés dans (1) le cycle de l'azote des biofilms et donc, dans les fonctions relatives à leur contribution aux processus « d'auto-épuration » des cours d'eau, et (2), dans la capacité de résistance des écosystèmes hyporhéiques face aux perturbations chimiques.

6.2 Version anglaise

The main finding of the thesis is that high density of rotifers can contribute to enhance nitrogen uptake capacity of (1) phototrophic biofilms (Chapters 2 and 3), and (4) hyporheic biofilms (Chapter 4) showing that meiofauna can play a significant role in nitrogen exchanges between the water column and biofilms. It appears that this contribution can be observed under relatively high $N\text{-}NO_3$ concentrations (in the phototrophic biofilm, 2.03 ± 0.004 mg L^{-1} and 2.70 ± 0.11 mg L^{-1} Chapters 2 and 3) mimicking downstream eutrophic conditions in the Garonne river. This effect of meiofauna was also observed under higher concentrations (in the hyporheic biofilm, 9.34 ± 1.19 mg L^{-1}) mimicking the hyporheic zone where nitrate concentrations can be higher than that in surface water (e.g. Krause et al., 2013) (Chapter 4). Moreover, results of Chapter 5 indicate that invertebrates can also diminish the perturbation by a toxic, diuron, manifested through a stimulated nitrate uptake by biofilms under herbicide exposition.

Rotifer and bacterial densities responded concomitantly to nutrient enrichments (Chapter 2) or were highly correlated (Fig. 3-3 and 3-4, Chapter 3) in nutrient enriched microcosms. Chapter 4 reports a possible link between invertebrate density and higher denitrification rates in the heterotrophic biofilms. Overall, these experiments strongly suggest that the positive influence of rotifers on the nitrate uptake of biofilms issued from interactions between rotifers and bacteria in the benthic zone as well as in the hyporheic zone. Since a number of interactions could occur between meiofauna and bacteria (trophic or indirect relationships), as discussed in (Chapter 3), further investigations are needed to examine their respective implication.

The meiofaunal effect on biofilm N uptake was observed under controlled conditions (e.g. stable river flow) in the present experiments. Therefore, the question that "can this in-laboratory meiofauna effect on biofilm N uptake occur in the field?" should be raised. Firstly, the $N\text{-}NO_3$ concentrations in the present study fell in the range from 0.66 ± 0.23 mg L^{-1} to 10.2 ± 1.9 mg L^{-1} occurring in the Garonne River (Iribar et al., 2007; Majdi et al., 2012a). Secondly, as shown in Fig. 6-2, for the Garonne river (Majdi et al. 2012a), during the relatively stable period (2009/08/04 – 2009/10/04), where biofilms growth, meiofaunal density is often dominated by rotifers. Based on these two facts, it can be assumed that the developments of such biofilm-associated meiofauna could probably play a role on biofilm N uptake in the field. However, the mean rotifer density in the present study (5.4 ± 1.3 10^3 ind

cm^{-2}, chapter 3) was higher than that in the field during the non-disturbed period (on average $0.06 \pm 0.03\ 10^3$ ind cm^{-2}). Thus, these results deserve carefully extrapolation.

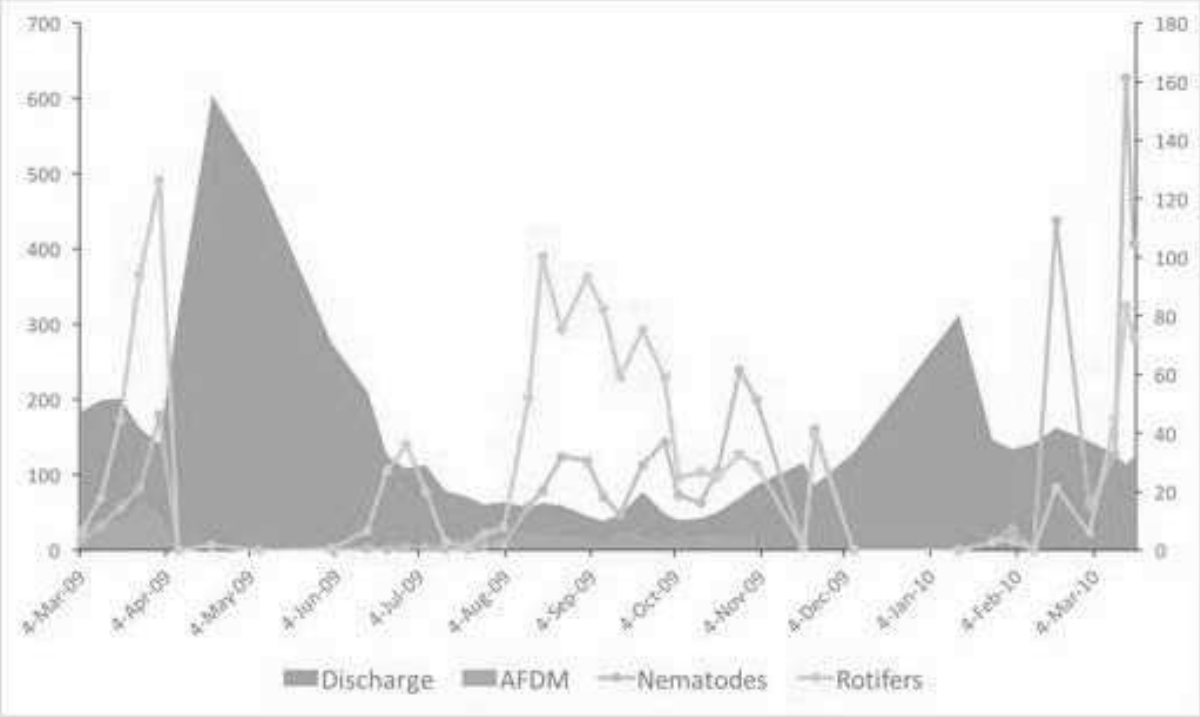

Figure 6-2 The densities of nematodes and rotifers (ind. cm^{-2}), discharge ($m^3\ s^{-1}$) and biofilm biomass (AFDM g m^{-2}) in Garonne River during a period from 2009/03/04 to 2010/03/04 after Majdi et al. 2012a

No significant change in composition and biomass of algae was observed, so, no evidence of the potential role of interactions with microalgae was found during the experiments. This was unexpected particularly for phototrophic biofilms, which generally contain an abundant microalgal community that accounts for a large part of the total biomass of mature biofilms (e.g. Barranguet et al., 2005). A first possible explanation is that, as explained in in Chapters 2 and 3, the lack of response of the microalgal biomass to nutrient enrichment was due to the ambient nitrate concentrations which were above the growth-limiting level for freshwater benthic algae (Stevenson et al., 1996). But also, it is possible that the extra primary production was grazed by the meiobenthos maintaining the microalgal biomass at a stable level, since rotifers are efficient grazers in phototrophic biofilms (Mialet et al., 2013). It is known that meiofaunal grazing can stimulate bacterial growth (Traunspurger et al., 1997; Hakenkamp & Morin, 2000), so, microalgal growth could have been also stimulated by grazing. This would suggest that these microalgae-meiofauna interactions could contribute to the observed increase in $N\text{-}NO_3$ uptake. Besides, Proia et al.

(2012) suggest that biofilm associated microalgae can increase their interactions with bacteria when their biomasses are not significantly enhanced by high nutrient exposure. In the present experiments, activity of algae was not measured and thus the question remains whether the meiofauna - bacteria - microalgae interactions would also significantly influence the nitrogen uptake by biofilms. A first indication could however be provided by comparing the increase of nitrate uptakes between phototrophic biofilms (Chapter 3) and heterotrophic biofilms (Chapter 4).

Table 6-1. Comparison of parameters and results between experiments in Chapter 3 and Chapter 4. N-NO_3: mean initial concentration (± SD) used in the nutrient enriched microcosms. Uptake increase: difference of mean N-NO_3 uptakes measured for biofilms with low and high density of rotifers* (Chapter 3), or, measured with and without meiofauna (Chapter 4).**

	Chapter 3	Chapter 4
Biofilms	Phototrophic (benthic)	Heterotrophic (hyporheic)
Substrates	Plexiglas	Sediments
Lights	Yes	No
Temperature (°C)	17	15
Flow rates (mL min^{-1})	2500	7.5
Time frames (days)	5	7
N-NO_3 concentration (mg L^{-1})	2.70 ± 0.11	9.34 ± 1.19
Uptake increase (%)	58.2	60.5
Meiofauna (10^3 ind. cm^{-2} ± SD)	5.4 ± 1.3*	1.0 ± 0.2**

Biofilm nitrate uptake rates in phototrophic biofilm after 2 days of 2.70 ± 0.11 mg L^{-1} nitrate addition (Chapter 3) increased 58.2 % in the treatments with high meiofauna than those with low meiofauna. Meanwhile, in heterotrophic biofilm after 7 days of 9.34 ± 1.19 mg L^{-1} nitrate addition (Chapter 4), biofilm nitrate uptake rates are increased 60.5 % in the treatments with meiofauna compared to those without meiofauna (Table. 6-1). So, there was no difference of nitrate uptake rates between phototrophic biofilm and in heterotrophic biofilm, although the conditions differed (shorter time frame, higher temperature, faster flow rates and lower initial nitrate concentration Table. 6-1). It is admitted that the above factors are also crucial to N cycle in aquatic systems thus, this observation must be considered with precaution. Nevertheless, this does not support the influence of microalgal activity. This is in line with results reported by Stock et al. (2014) showing that microalgae (diatoms) alone had no effect on N fluxes in marine sediments, but that they did enhance the effect of meiofauna (copepods) on dissimilatory nitrate reduction for seven and a half day incubation in

microcosms. The excretion of meiofauna could favor the bacterial growth (e.g. Riemann & Helmke, 2002). As such, possible interactions among meiofauna, algae and bacteria could be established in the sense that microalgae could indirectly enhance bacteria growth through meiofauna excretion products resulting from their feeding activity (Stock et al., 2014).

In Chapters 4 and 5, macrofauna groups were added into the experiment design to achieve a higher cross-community diversity. This showed that increasing biodiversity enhanced $N\text{-}NO_3$ and DOC uptake rates in hyporheic zones. It must be noticed that the improvement of nitrogen uptake by meiofauna enrichment was again increased in the treatments with the co-occurrence of macrofauna (mainly chronomidae larvae) and meiofauna, in the experiment which did not use herbicide exposition (Chapter 4). Besides, macrofauna has been previously reported to decrease both meiofauna activity and abundance in sediments due to disturbance, predation or competition for food (Alongi, 1985; Branch & Pringle, 1987; Olafsson, 2003). Bonaglia et al. (2014) have shown that the bivalve *Macoma balthica*, counteracted the stimulating effect for the nitrifying and denitrifying microbial communities by meiofauna in marine sediments. As cited by Bonaglia et al. (2014), this probably resulted from interference competition with meiofauna for freshly deposited organic matter as previously suggested (Nascimento et al., 2011). The results described in Chapter 4 contrasted with these observations. Chironomid larvae belong to benthic infauna in freshwater sediments that act as ecosystem engineers (Stief, 2013). They are known to stimulate sedimentary nitrification and denitrification in lakes by irrigating their burrows intermittently (e.g. Pelegrí & Blackburn, 1996; Svensson, 1997; 1998; Lewandowski et al., 2007; Roskosch et al., 2010; Stief, 2013). Moreover, it has been shown that the density of nitrogen-cycling bacteria is higher in the burrow walls than in the sediment surrounding the burrows (Satoh et al., 2007). It is thus conceivable that additionally to the intermittent ventilation effect, chironomid larvae burrows stimulated growth of bacteria and thus possibly interactions between bacteria and meiofauna during the experiment reported in Chapter 4. Results highlight that further investigations of effects of macrofauna-meiofauna-bacteria interactions on N transformation processes are needed since it is still largely unexplored (Bonaglia et al. 2014), and such interactions should be investigated to improve our understanding of nitrogen uptake processes by biofilms.

The positive effect of rotifers on nitrogen uptake capacity of biofilms is supported by their short-term increase in density and biomass in response to nutrient enrichment (Chapter 2 experiment). This is in line with previous reports of the response of benthic rotifers to increasing phosphorus in lake habitats and in microcosm sediments (Särkkä, 1992; Ristau &

Traunspurger, 2011; Ristau et al., 2012; Wu et al., 2014) suggesting that the potential role of benthic rotifer assemblages as indicators of ecosystem trophic state should be investigated as was done for planktonic rotifers in lakes (Duggan et al., 2001).

The positive indirect effect of meiofauna, especially rotifers on biofilm nitrate uptake possibly through their interactions with bacteria is highlighted both in benthic and hyporheic zones of lotic ecosystems. As shown in Fig. 6-2, nematodes can also be often dominant meiofauna organisms in biofilms e.g. 20-319 ind. cm^{-2} (Majdi et al., 2012a), but were not relatively abundant in the present experiments (0.3-10 ind. cm^{-2} in Chapter 2, 0.2 % in Chapter 3, and 1.7 % in Chapter 4). In the present experiments, it was not observed that nematode density could react to moderate nutrient inputs in our 5-day experiment (Chapter 2). Nevertheless, Gaudes et al. (2012) found that the density of nematodes from sandy samples in Mediterranean forested streams can be positively affected by nutrient addition during a 2-year period. This suggests that reaction of nematodes to nutrient input could take more time to be notable than that of rotifers. Majdi et al. (2012a) showed that nematode development in Garonne phototrophic biofilms is more slow than this of rotifers, which are the pioneer taxa after a destruction and redevelopment of the biofilm. Besides, it is known that nematodes can (1) assimilate bacteria and algae (e.g. Moens et al., 2006), (2) stimulate bacteria growth by mucus secretions (Riemann & Helmke, 2002) and (3) modify oxygen turn over and primary productivity of biofilms (Mathieu et al., 2007). Thus, it is likely that nematodes can influence on biofilms nitrate uptake through their interactions with bacteria and algae. This should be considered and incorporated into further investigations. Moreover, as it was suggested for marine coastal sediments by Bonaglia et al. (2014), it must be noticed that others biofilms associated groups which are potentially involved in regulating bacteria-mediated processes, such as protozoans, have been so far underexplored for their contribution to these biofilm processes.

Whereas benthic macrofauna has been described to negatively affect biofilm processes related to their nutrient uptake ability (e.g. Sabater et al., 2002), the present experiments highlight that in contrast, meiofauna can positively affect $N\text{-}NO_3$ uptake in both phototrophic and autotrophic biofilms through their interactions with micro-organisms and macrofauna and reduce the perturbation (manifested as an increase) created in $N\text{-}NO_3$ by the addition of a toxic to the microcosm. The rapid response of rotifers to nutrient addition added to their better resistance to flow disturbance than nematodes (Majdi et al., 2012a) indicates that biofilm associated rotifers could be particularly considered for development of ecological state indicator of lotic ecosystems and water quality amelioration studies. This has been

previously suggested for nematodes in Mediterranean coastal ecosystems (Moreno et al., 2011) and in estuarine ecosystems (Patrício et al., 2012; Alves et al., 2013) but to our knowledge, so far, it has not been studied for lotic meiobenthic rotifers. It is particularly emphasized that the biofilm depuration processes could be enhanced by increasing cross-community diversity. These results suggest that on and under the riverbed, all associated biological compartments could actively participate in the water quality amelioration process, and support the general hypothesis that biodiversity contributes positively to ecosystems process (Loreau et al., 2001).

This thesis highlights that meiofauna may play a significant role in nitrogen consumption processes by lotic biofilms. In addition, the results strongly suggest that invertebrates interact with microorganisms involved in the reduction processes of nitrogen concentrations in the phototrophic biofilm as well as the hyporheic biofilm. Finally, the herbicide exposure resulted in a significant modification of $N\text{-}NO_3$ uptake rate in hyporheic microcosms. However, the comparison of the average $N\text{-}NO_3$ uptake rate between treatments exposed to herbicide and those unexposed, showed that the presence of invertebrates (meiofauna + macrofauna) significantly reduced the effect of diuron on these processes. This study highlights the potentially important role of microorganism-invertebrate interactions (1) in the nitrogen cycle of biofilms and thus functions related to their contribution to the "self-depuration" process in streams, and (2) in resilient capacity of the hyporheic ecosystem to chemical perturbations.

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Résumé

Le rôle de la méiofaune sur le fonctionnement des biofilms lotiques a été examiné par l'étude de son effet potentiel sur la capacité de consommation de l'azote des biofilms, au cours de quatre expérimentations. Les deux premières (Chapitres 2 et 3) concernent les biofilms épilithiques (phototrophes) tandis que les deux autres ont porté sur les biofilms (hétérotrophes) de la zone hyporhéique. Les biofilms sont soumis (1) à différents niveaux de densités (méiofaune) et à un enrichissement en nutriments ou (2) à différents niveaux de diversité (biofilm/méiofaune/macrofaune). Une partie des microcosmes présentant chaque niveau de diversité a été exposée à l'effet d'un herbicide, le diuron. Dans l'ensemble, la méiofaune associée aux biofilms des microcosmes était largement dominée par les rotifères. Les résultats basés sur les biofilms phototrophes montrent que les rotifères peuvent répondre à court terme, à un enrichissement en $N\text{-}NO_3$ par une augmentation significative de leur densité et biomasse. De plus, le taux de consommation de $N\text{-}NO_3$ est apparu significativement plus élevé dans les microcosmes dont les densités de méiofaune étaient les plus élevées. Cet effet positif de la méiofaune sur la consommation de $N\text{-}NO_3$ par les biofilms a été retrouvé dans l'étude basée sur le biofilm hyporhéique pour les microcosmes non soumis aux effets du diuron. Dans l'ensemble, ce travail met donc en évidence le rôle significatif que peut avoir la méiofaune dans les processus de consommation de l'azote par les biofilms lotiques. De plus, les résultats suggèrent fortement que les invertébrés interagissent avec les micro-organismes impliqués dans les processus de réduction des concentrations en azote, dans le biofilm phototrophe comme dans le biofilm hyporhéique. Enfin, l'exposition à l'herbicide a engendré une modification significative du taux de consommation de $N\text{-}NO_3$ dans les microcosmes hyporhéiques. Cependant, la comparaison du taux de consommation moyen de $N\text{-}NO_3$ entre les traitements exposés à l'herbicide et ceux non exposés, a montré que la présence des invertébrés (méiofaune + macrobenthos) a significativement réduit l'effet du diuron sur ces processus. Cette étude met en exergue le rôle potentiellement important des interactions micro-organismes – invertébrés dans (1) le cycle de l'azote des biofilms et donc, dans les fonctions relatives à leur contribution aux processus « d'auto-épuration » des cours d'eau, et (2), dans la capacité de résistance des écosystèmes hyporhéiques face aux perturbations chimiques.

Mots clés: méiofaune, rotifères, biofilms, bactéries, microphytobenthos, interactions, azote, consommation des nitrates, eutrophisation, dénitrification, diversité, diuron, herbicide, cours d'eau

Abstract

The role of meiofauna on the functioning of riverine biofilms was examined by studying their potential effect on nitrogen consumption capacity of biofilms in four experiments (Chapters 2 and 3: epilithic phototrophic biofilms; Chapters 4 and 5: heterotrophic biofilms of hyporheic zone). Biofilms are subjected to (1) different levels of densities (meiofauna) and nutrient enrichment or (2) different levels of diversity (biofilm/meiofauna/macrofauna). A part of the microcosms of each level of diversity was exposed to the effect of an herbicide, diuron. Overall, biofilm-associated meiofauna in microcosms was dominated by rotifers. Results in phototrophic biofilms showed that the response of rotifers to short-term nutrient enrichment was significant increases in their density and biomass. In addition, $N\text{-}NO_3$ uptake rates appeared significantly higher in microcosms with highest meiofauna densities. This positive effect of meiofauna on biofilm $N\text{-}NO_3$ uptake was also found in hyporheic biofilm microcosms, but not under the effect of diuron. Therefore, this thesis highlights that meiofauna can have a significant role in nitrogen consumption processes by lotic biofilms. In addition, the results strongly suggest that invertebrates interact with microorganisms involved in the reduction processes of nitrogen concentrations in the phototrophic biofilm as well as the hyporheic biofilm. Finally, the herbicide exposure resulted in a significant modification of $N\text{-}NO_3$ uptake rate in hyporheic microcosms. However, the comparison of the average $N\text{-}NO_3$ uptake rate between treatments exposed to herbicide and those unexposed, showed that the presence of invertebrates (meiofauna + macrofauna) significantly reduced the effect of diuron on these processes. This study highlights the potentially important role of microorganism-invertebrate interactions (1) in the nitrogen cycle of biofilms and thus functions related to their contribution to the "self-purification" process in streams, and (2) in resilient capacity of the hyporheic ecosystem to chemical perturbations.

Keywords: meiofauna, rotifers, biofilm, bacteria, microphytobenthos, interactions, nitrogen, nitrate uptake, eutrophication, denitrification, diversity, diuron, herbicide, watercourse